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**Mass Spectral Confirmation of Chlorinated
and Brominated Diphenylethers in
Human Adipose Tissues**

Final Report

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PREFACE

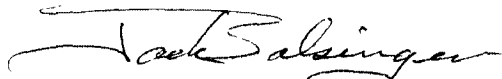
This report provides a summary of the work completed to confirm and quantitate, inasmuch as available standards would allow, the level of polychlorinated and polybrominated diphenylethers (PCDPEs and PBDPEs) in human adipose tissues. The samples analyzed were previously prepared from selected FY87 NHATS specimen composites that were analyzed for polychlorinated and polybrominated dibenzo-*p*-dioxins and dibenzofurans. The results from the determination of the halogenated dibenzo-*p*-dioxins and dibenzofurans are presented in separate reports. This work was conducted under EPA Contract No. 68-02-4252, Work Assignment 27, "Analysis of Human Adipose Tissue for Dioxins and Furans."

The data and reporting activities were generated by Midwest Research Institute (MRI) under the direction of Mr. Paul H. Cramer and Dr. John S. Stanley for EPA's Office of Toxic Substances, Field Studies Branch. Mr. Kelly Thornburg conducted the HRGC/HRMS analysis.

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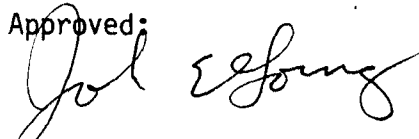

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GLOSSARY

BCD	Battelle Columbus Division
DPE	Diphenylether
BDPE	Decabromodiphenylether
DCDPE	Decachlorodiphenylether
EED	Exposure Evaluation Division
EPA	Environmental Protection Agency
FY	Fiscal year
HDPE	Halogenated diphenylether
HpBDD	Heptabromodibenzo- <i>p</i> -dioxin
HpBDF	Heptabromodibenzofuran
HpBDPE	Heptabromodiphenylether
HpCDD	Heptachlorodibenzo- <i>p</i> -dioxin
HpCDF	Heptachlorodibenzofuran
HpCDPE	Heptachlorodiphenylether
HxBDD	Hexabromodibenzo- <i>p</i> -dioxin
HxBDF	Hexabromodibenzofuran
HxBDPE	Hexabromodiphenylether
HxCDD	Hexachlorodibenzo- <i>p</i> -dioxin
HxCDF	Hexachlorodibenzofuran
HxCDPE	Hexachlorodiphenylether
IQS	Internal quantitation standard
MRI	Midwest Research Institute
MSA	Metropolitan Statistical Area
NBDPE	Nonabromodiphenylether
NCDPE	Nonachlorodiphenylether
NHATS	National Human Adipose Tissue Survey
OBDD	Octabromodibenzo- <i>p</i> -dioxin
OBDF	Octabromodibenzofuran
OBDE	Octabromodiphenylether
OCDD	Octachlorodibenzo- <i>p</i> -dioxin
OCDF	Octachlorodibenzofuran
OCDE	Octachlorodiphenylether
OTS	Office of Toxic Substances
PBDD	Polybrominated dibenzo- <i>p</i> -dioxin
PBDF	Polybrominated dibenzofuran
PBDPE	Polybrominated diphenylether
PCBs	Polychlorinated biphenyls
PCDD	Polychlorodibenzo- <i>p</i> -dioxin
PCDF	Polychlorodibenzofuran
PCDE	Polychlorinated dibenzofuran
PCDE	Polychlorinated diphenylether
PeBDD	Pentabromodibenzo- <i>p</i> -dioxin
PeBDF	Pentabromodibenzofuran
PeCDD	Pentachlorodibenzo- <i>p</i> -dioxin
PeCDF	Pentachlorodibenzofuran
PHDD	Polyhalogenated Dibenzo- <i>p</i> -dioxin
PHDF	Polyhalogenated Dibenzofuran
PHDPE	Polyhalogenated Dibenzodiphenylether
RS	Recovery standard
TBDD	Tetrabromodibenzo- <i>p</i> -dioxin
TBDF	Tetrabromodibenzofuran
TCDD	Tetrachlorodibenzo- <i>p</i> -dioxin
TCDF	Tetrachlorodibenzofuran
TSCA	Toxic Substances Control Act

EXECUTIVE SUMMARY

This study has resulted in the detection and confirmation of polyhalogenated diphenylethers (PHDPEs) in human adipose tissues. The PHDPEs are of interest because of their planar aromatic structure and potential toxicological properties. The PHDPEs are commercially produced as brominated fire retardants and are known contaminants in some other commercial products, e.g., pentachlorophenol. The samples that were analyzed were selected from composites of the fiscal year 1987 National Human Adipose Tissue Survey (FY87 NHATS) repository. The identifications were based on both full scan and selected ion monitoring (SIM) high resolution mass spectrometry (HRMS).

This confirmation study was conducted as a result of responses to the PHDPE compounds observed during the preliminary analysis of the FY87 NHATS composites for polyhalogenated dibenzo-*p*-dioxins and dibenzofurans (PHDDs/PHDFs). Concentrations of the PHDPEs were estimated from the preliminary analysis efforts, which focused on the determination of PHDDs and PHDFs. The analysis efforts for PCDPEs and PBDPEs summarized in this report were conducted using standard solutions of several compounds and compound mixtures. Identification of the PHDPEs was based on comparison of full scan mass spectra of the samples to the available standards, application of SIM techniques to compare theoretical ion ratios to observed ion ratios for characteristic ions, and measurement of fragment losses from the molecular ion clusters. Data pertaining to estimated concentrations, and tentative isomer identification were achieved through comparison of responses from the analysis of a PCDPE standard solution, and the results of the analysis of two commercial fire retardants and the analysis of a decabrominated diphenylether standard.

Briefly, the approach was as follows. Four samples were analyzed by full scan GC/MS. Both nonachloro- and hexabromodiphenylethers were observed under the full scan conditions. Five samples were analyzed for PCDPEs and five for PBDPEs by high resolution SIM.

All samples were found to contain detectable levels of the PHDPEs. The response profiles or patterns for both the chlorinated and brominated diphenylethers were consistent across all samples regardless of age or geographic region. Analysis of laboratory method blanks that had been prepared with the FY87 NHATS samples demonstrated no contribution from laboratory background.

The values calculated in this confirmation study versus the authentic PCDPE standards are comparable with the estimates of the PCDPE levels from the preliminary analysis effort. Hence, the data generated using the PCDF RRF measurements are considered good preliminary values of levels in the general U.S. population. Based on the comparison of results between the two analysis efforts, it is possible to extrapolate approximate or estimated concentrations of these compounds in the FY87 NHATS analysis effort. The levels of PCDPEs reported, however, have a number of caveats: the sample preparation procedures are not optimized for PCDPE recoveries and the quantitations are based on recoveries of carbon 13-labeled PCDF internal

quantitation standards. The sample preparation scheme was originally developed to minimize interferences from these compounds. Previous attempts by other researchers to determine the PCDPE levels in the adipose tissues of the Canadian population were not successful in identifying these compounds but demonstrated that if present the concentrations were less than 10 ng/g (ppb).

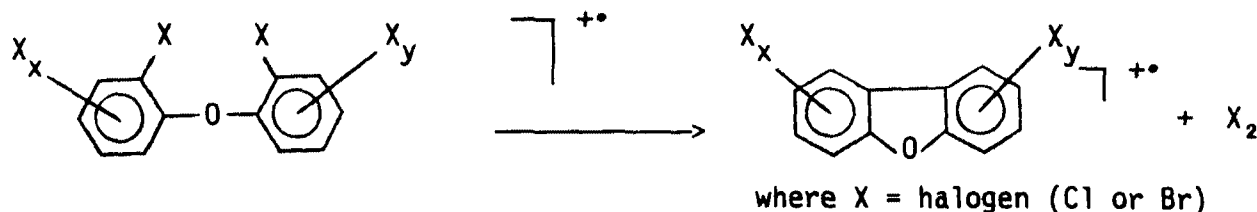
Although the presence of the PBDPEs was confirmed by the additional HRMS-SIM experiments, it was not possible to confirm the estimates of concentrations from the preliminary effort due to a lack of individual PBDPE isomers. In addition to confirming the presence of the hexa- through octabromodiphenylethers, nonabromo and decabromodiphenylethers were identified. The presence of decabromodiphenylether was identified in three of the five extracts analyzed at concentrations ranging from approximately 400 to 700 pg/g based on an external standard response.

Further evaluation of the levels of halogenated aromatic compounds in human adipose tissues should include refinement of analytical methods to promote simultaneous determination of dioxins, furans, diphenylethers, and non-ortho-substituted biphenyls. Refinement of the analytical methods will require development and acquisition of additional analytical standards and internal quantitation standards to promote quantitative recovery and precision. To provide indications of routes of exposure, the data resulting from further studies should be analyzed for correlations between compound classes. Additional analysis efforts for PBDPEs should also include experiments to determine the presence of other PBDPEs such as the tetra- and pentabromo congeners.

I. INTRODUCTION

The U.S. Environmental Protection Agency (EPA) has promulgated regulations under Sections 4 and 8 of the Toxic Substances Control Act (TSCA) for chemicals that may be contaminated with polyhalogenated dioxins (PHDDs) and furans (PHDFs).¹ As part of EPA's effort to assess the potential exposure of humans to these compounds, human adipose tissues collected in FY87 through the EPA's National Human Adipose Tissue Survey (NHATS) were analyzed for both chlorinated and brominated (halogenated) dibenzo-*p*-dioxins and dibenzofurans.²⁻⁴

The analysis protocol for the PHDDs and PHDFs required monitoring ions characteristic of halogenated diphenylethers (HDPEs) simultaneously with ions for the PHDDs and PHDFs via high resolution gas chromatography/high resolution mass spectrometry (HRGC/HRMS). The data generated demonstrated that the HDPEs yield fragment ions with the same exact masses and characteristic ion ratios observed for PHDFs. The unique structure of the halogenated diphenylethers is such that the loss of two halogen atoms from the molecular ions in the mass spectrometer ion source can yield radical cations that are indistinguishable in mass from those produced by PCDF and PBDF compounds. A possible mechanism for this reaction in the mass spectrometer is given below.



The presence of HDPEs in the adipose tissue samples was indicated by monitoring the molecular ion region for hexa- through decahalogenated diphenylethers during the determination of tetra- through hexabrominated and tetra- through octachlorinated dibenzo-*p*-dioxins and dibenzofurans. Preliminary estimates of the levels of the HDPEs were achieved by using calibration data generated for the halogenated dibenzofurans. Tables 1 and 2 present the estimated levels of the chlorinated and brominated DPEs for the specific FY87 NHATS composites analyzed.⁴

The quantitative measurements presented in this report were limited by the availability of authentic, certified standards of HDPEs. Primary standards of individual HDPEs were not commercially available at the time of this study. The chlorinated diphenylether compounds were obtained as a solution of several compounds from Dr. D. T. Williams of Health and Welfare Canada. The brominated diphenylethers were obtained as mixtures of brominated fire retardants (Bromkal 70-5-DE and 79-8-DE, Ultra Scientific). Decabromodiphenylether was available as a neat standard from a commercial source (Ultra Scientific). The limited availability of analytical standards and the lack of isotopically labeled analogs for use as internal standards for the diphenylethers made it difficult to quantitate the exact amounts of diphenylethers detected in the adipose tissue extracts.

Table 1. Estimated Polychlorinated Diphenylether (PCDPE)
Lipid Concentrations in NHATS FY87 Composites

Composite # FY87 (NHATS)	Census Division (a)	Age Group	Estimated Concentration (pg/g) (b)				
			HxCdPE	HpCdPE	OCDPE	NCdPE	DCdPE
ACD8700014	EN	0 - 14	ND (c)	4	20	200	ND
ACD8700023	EN	0 - 14	1	2	10	200	20
ACD8700032	EN	15 - 44	1	4	100	800	5
ACD8700041	EN	15 - 44	4	5	80	800	ND
ACD8700050	EN	15 - 44	4	4	40	500	ND
ACD8700069	EN	45 +	9	6	100	1000	ND
ACD8700078	EN	45 +	10	10	200	900	ND
ACD8700087	EN	45 +	5	10	200	1000	ND
ACD8700096	EN	45 +	5	6	100	800	ND
ACD8700103	EN	45 +	7	5	100	1000	ND
ACD8700112	ES	0 - 14	1	2	10	80	ND
ACD8700121	ES	15 - 44	3	3	90	900	ND
ACD8700130	ES	45 +	6	5	100	1000	10
ACD8700149	MA	0 - 14	2	ND	10	200	8
ACD8700158	MA	15 - 44	2	3	70	700	8
ACD8700167	MA	15 - 44	4	7	100	1000	ND
ACD8700176	MA	15 - 44	3	4	60	800	ND
ACD8700185	MA	45 +	5	5	200	1000	10
ACD8700194	MA	45 +	5	5	200	1000	ND
ACD8700201	MO	0 - 14	ND	ND	5	50	6
ACD8700210	MO	15 - 44	1	1	60	800	ND
ACD8700229	MO	45 +	4	4	200	800	ND
ACD8700238	NE	0 - 14	ND	ND	ND	30	ND
ACD8700247	NE	15 - 44	2	10	40	200	20
ACD8700256	NE	45 +	20	70	100	600	ND
ACD8700265	PA	0 - 14	1	ND	ND	30	ND
ACD8700274	PA	15 - 44	3	2	30	500	ND
ACD8700283	PA	45 +	10	5	200	1000	ND
ACD8700292	PA	45 +	6	5	200	900	ND
ACD8700309	PA	45 +	5	4	100	900	ND
ACD8700318	SA	0 - 14	2	ND	10	100	ND
ACD8700327	SA	0 - 14	3	ND	10	100	ND
ACD8700336	SA	15 - 44	7	8	100	1000	ND
ACD8700345	SA	15 - 44	7	6	80	800	7
ACD8700354	SA	15 - 44	2	3	80	900	ND
ACD8700363	SA	15 - 44	8	4	30	400	ND
ACD8700372	SA	45 +	3	7	100	2000	9
ACD8700381	SA	45 +	4	3	100	1000	ND
ACD8700390	SA	45 +	9	8	200	900	ND
ACD8700407	SA	45 +	10	10	200	900	ND
ACD8700416	WN	0 - 14	1	ND	3	100	ND
ACD8700434	WN	15 - 44	2	5	80	900	ND
ACD8700443	WN	45 +	2	2	100	700	ND
ACD8700452	WN	45 +	10	10	200	2000	ND
ACD8700461	WS	0 - 14	3	3	20	200	ND
ACD8700470	WS	15 - 44	4	5	50	600	ND
ACD8700489	WS	45 +	3	3	100	900	ND

- (a) - EN = East North Central, ES = East South Central, MA = Middle Atlantic, MO = Mountain,
NE = New England, PA = Pacific, SA = South Atlantic, WN = West North Central, WS = West South Central.
(b) - Approximate PCDPE concentration calculated by using corresponding PCDF relative response factors.
(c) - Not detected.

Table 2. Estimated Polybrominated Diphenylether Lipid Concentrations
in NHATS FY87 Composites

Composite # FY87 (NHATS)	Census Division (a)	Age Group	Estimated Concentration (pg/g) (b)		
			HxBDPE	HpBDPE	OBDPE
ACD8700014	EN	0 - 14	4	300	8000
ACD8700023 (d)	EN	0 - 14	700	200	800
ACD8700032	EN	15 - 44	10	70	ND (c)
ACD8700041	EN	15 - 44	ND	100	700
ACD8700050	EN	15 - 44	ND	200	600
ACD8700069	EN	45 +	200	90	200
ACD8700078	EN	45 +	1000	60	ND
ACD8700087	EN	45 +	300	200	600
ACD8700096	EN	45 +	500	200	400
ACD8700103 (e)	EN	45 +	200	200	400
ACD8700112	ES	0 - 14	8	200	ND
ACD8700121	ES	15 - 44	30	200	ND
ACD8700130	ES	45 +	900	400	3000
ACD8700149	MA	0 - 14	ND	50	300
ACD8700158	MA	15 - 44	20	200	ND
ACD8700167	MA	15 - 44	600	300	3000
ACD8700176	MA	15 - 44	400	300	2000
ACD8700185	MA	45 +	300	50	ND
ACD8700201	MO	0 - 14	100	1	ND
ACD8700210	MO	15 - 44	ND	100	400
ACD8700229	MO	45 +	ND	3	ND
ACD8700238	NE	0 - 14	ND	30	ND
ACD8700247	NE	15 - 44	200	200	ND
ACD8700256	NE	45 +	ND	50	ND
ACD8700265	PA	0 - 14	500	2000	3000
ACD8700274	PA	15 - 44	2	100	ND
ACD8700283	PA	45 +	ND	80	ND
ACD8700292	PA	45 +	200	40	200
ACD8700309	PA	45 +	ND	100	ND
ACD8700318 (d)	SA	0 - 14	300	200	800
ACD8700327	SA	0 - 14	10	70	ND
ACD8700336 (d)	SA	15 - 44	500	400	2000
ACD8700345	SA	15 - 44	ND	300	ND
ACD8700354	SA	15 - 44	7	100	600
ACD8700363	SA	15 - 44	9	200	100
ACD8700372	SA	45 +	ND	200	ND
ACD8700381	SA	45 +	300	100	400
ACD8700390	SA	45 +	200	40	200
ACD8700407 (d)	SA	45 +	600	100	1000
ACD8700416	WN	0 - 14	600	90	100
ACD8700425	WN	15 - 44	ND	100	400
ACD8700434	WN	15 - 44	10	400	ND
ACD8700443	WN	45 +	900	30	70
ACD8700452	WN	45 +	ND	200	ND
ACD8700461	WS	0 - 14	20	200	100
ACD8700470	WS	15 - 44	5	100	600
ACD8700489	WS	45 +	700	100	400

(a) - EN = East North Central, ES = East South Central, MA = Middle Atlantic, MO = Mountain,

NE = New England, PA = Pacific, SA = South Atlantic, WN = West North Central, WS = West South Central.

(b) - PBDPE concentration calculated by using corresponding PBDF relative response factors.

(c) - Not detected.

(d) - The HRMS confirmational analysis described in this report includes the determination of nona- (NBDPE) and (DBDPE) decabromodiphenylethers. Two responses were detected for NBDPE but were not quantitated due to the lack of an appropriate standard. The DBDPE was estimated at 400 pg/g and 700 pg/g based on an external standard for samples ACD8700336 and ACD8700407, respectively. The DBDPE was noted as a weak response in sample ACD8700023. The DBDPE was not detected in sample ACD8700318.

(e) - Analysis was conducted for DBDPE but was not detected.

The following sections of this report are organized as follows. Section II states the objectives of the study. Section III discusses the technical approach used to verify the occurrence of diphenylethers in adipose tissue. Section IV presents the experimental details. Section V presents the results of the GC/MS analyses. Sections VI and VII present the conclusions and recommendations, respectively.

II. OBJECTIVES

The primary objective of this study was to confirm the presence of brominated and chlorinated diphenylether compounds in the FY87 NHATS adipose tissue extracts. To accomplish this objective, selected extracts were analyzed by mass spectrometry using both full scan and selected ion monitoring modes. The secondary objective of this study was to compare the concentrations of the chlorinated DPE calculated using authentic standards to the originally estimated concentrations using the PCDF response factor values (Table 1). Further confirmation of the levels of brominated diphenylethers was not possible due to the lack of authentic individual standards.

III. TECHNICAL APPROACH

The approach to confirmation of the polychlorinated and polybrominated diphenylethers was based on generating additional mass spectral information using both HRGC/MS in the full scan mode and HRGC-HRMS via selected ion monitoring (SIM). The data generated by these two techniques support compound identification by providing characterization of the fragmentation patterns, ion ratios, and the exact masses of the compounds under investigation.

A subset of extracts from the FY87 NHATS composites were selected for confirmation of identification and previous quantitative efforts. Table 3 gives the overall analytical scheme for confirmation of PBDEs and PCDEs. Each sample is identified by a composite number code, and the batch number in which the samples were originally prepared is specified.

A total of 12 of the original 48 composite sample extracts were selected for confirmational analyses based on the previous estimates of concentration from the PCDD/PCDF and PBDD/PBDF analyses (Tables 1 and 2). Confirmational analyses of each sample via both full scan and SIM techniques were not possible due to limited volumes of the sample extracts. The original volume prior to analysis for PHDDs and PHDFs was 10 μ L. The determination of the PHDDs and PHDFs required two separate analyses (of 1 to 2 μ L each) for the brominated versus chlorinated congeners. Hence, the final volume available for confirmational analysis was only 6 to 8 μ L.

The preparation of the composite FY87 NHATS adipose tissue samples for analysis of PHDDs and PHDFs has been described in previous reports.²⁻⁴ Additional sample preparation was not necessary for the confirmational analyses discussed in this report.

Table 3. Overall Analytical Scheme for Confirmation of PBDPEs and PCDEs

Lab ID No.	Composite No.	Batch No.	Census Division	Age Group	Analysis Conducted		
					Full Scan	PCDPE - SIM	PBDPE - SIM
16251	-	1	-	-			X
16257	ACD8700023	1	EN	0-14			X
16258	ACD8700318	1	SA	0-14			X
16269	ACD8700283	2	PA	45+		X	
16278	-	3	-	-		X	
16279	ACD8700185	3	MA	45+		X	
16286	ACD8700381	3	SA	45+		X	
16289	ACD8700167	3	MA	15-44	X	X	
16294	ACD8700130	4	ES	45+	X		
16295	ACD8700103	4	EN	45+			X
16305	-	5	-	-	X		X
16309	ACD8700265	5	PA	0-14	X		
16313	ACD8700452	5	WN	45+		X	
16316	ACD8700336	5	SA	15-44	X		X
16317	ACD8700407	5	SA	45+			X

Samples with Lab ID nos. 16251, 16278, and 16305 are method blanks prepared in the respective batches.

IV. EXPERIMENTAL

The experimental details of this study are presented and discussed in the following sections.

A. Confirmation of Polychlorinated and Polybrominated Diphenylethers by Full Scan HRGC/MS

For the full scan analysis mode, a 30-m column was used so that both the chlorinated and brominated species could be confirmed in the same HRGC run. The sample extracts selected had some of the highest estimated PHDPE levels (Tables 1 and 2). Mass spectra obtained from the samples were compared to those from the available PCDE and PBDE standards and to the isomer patterns or molecular clusters predicted for multiple chlorinated or brominated species. Additional information on the characteristic spectra of PCDEs and PBDEs were identified in the literature.⁵⁻⁹ The HRGC/MS operation parameters for the full scan analysis are identified in Table 4.

B. Confirmation/Quantitation of Polychlorinated Diphenylethers (PCDEs) by HRGC/HRMS-SIM

In order to confirm and quantitate the presence of PCDEs, the HRGC/MS-SIM experiment was modified from that of the PCDD/PCDF protocol to increase the specificity for PCDEs without sacrificing overall sensitivity. Additional ions characteristic of PCDE molecular ion clusters were added to the quantitation list, and those ions normally monitored for the identification and quantitation of PCDDs were deleted. Because the total number of ions monitored was essentially the same as the number monitored during a normal PCDD and PCDF analysis, the sensitivity of the analysis for PCDFs and PCDEs was not compromised.

Three characteristic ions of the PCDE molecular ion cluster, three ions from the molecular ion cluster of the PCDFs (or indicative of the loss of two chlorines from the PCDE molecular ion cluster), and an ion representative of the loss of an additional fragment (COCl) were monitored along with the two characteristic ions from the ¹³C-PCDF internal quantitation standard. Fragment ions from PFK were also monitored to ensure correct mass assignment throughout the analyses.

Table 4 provides the HRGC/HRMS-SIM parameters used and Table 5 gives the ions monitored. The HRGC conditions identified in Table 4 are consistent with parameters used for determination of PCDDs and PCDFs. The data from the analyses were evaluated for coincidence of responses and correspondence of measured ion ratios to theoretical values.

PCDE levels were quantified by comparing responses to a standard of eight specific PCDEs. This standard was obtained as a solution from Dr. D. T. Williams of Health and Welfare Canada.¹⁰ The PCDE congeners are identified in Table 6. Table 6 also provides details on the composition of the four calibration standards. As noted, only five of the available PCDEs were used to calculate relative response factors (RRFs). The tetra- and pentachloro DEs were not included in the analysis strategies, and the 2,2',3,3',4,4'-HxCDE was outside the retention window of interest for this study.

Table 4. HRGC/MS Parameters

	Full scan	PCDPE-SIM	PBDPE-SIM
Mass spectrometer (VG-70-250S)			
Accelerating voltage:	8,000 V	8,000 V	8,000 V
Trap current:	500 μ A	500 μ A	500 μ A
Electron energy:	35 eV	35 eV	35 eV
Photo-multiplier voltage:	240 V	240 V	240 V
Resolution:	1000	> 10,000	> 10,000
Overall cycle time:	1.5 s	~ 1 s	~ 1 s
Mass range:	m/z 100-1000	m/z 243-516	m/z 375-961
Gas chromatograph (HP-5890)			
Column coating:	DB-5	DB-5	DB-5
Film thickness:	0.25 μ M	0.25 μ M	0.25 μ M
Column dimensions:	30 m x 0.25 mm	60 m x 0.25 mm	30 m x 0.25 mm
He linear velocity:	30 cm/s	30 cm/s	30 cm/s
Injection type:	splitless/split	splitless/split	splitless/split
Split flow:	30 mL/min	30 mL/min	30 mL/min
Purge flow:	3 mL/min	3 mL/min	3 mL/min
Injector temperature:	290	290	290
Interface temperature:	280	280	280
Injection size:	1 μ L	1 μ L	1 μ L
Initial temperature:	200	200	200
Initial time:	2 min	2 min	2 min
Temperature program:	b	a	b
Final hold time:	30 min	4 min	30 min

^aTemperature program--200-220°C at 5°C/min (16-min hold); then 5°C/min to 235°C (7-min hold); then 5°C/min to 330°C.

^bTemperature program--200-300°C at 5°C/min.

Table 5. Ions Monitored for the Determination of PCDFE

Descriptor 1 Accurate mass	Elemental composition	Ion ID	
		TCDF	HxCDFE
242.9394	$C_{11}H_4^{35}Cl_2^{37}Cl_1$	$[(M+2)-COCl]^+$	$[(M+2)-2Cl-COCl]^+$
303.9016	$C_{12}H_4O^{35}Cl_4$	$[M]^{+•}$	$[M-2Cl]^{+•}$
305.8987	$C_{12}H_4O^{35}Cl_3^{37}Cl_1$	$[M+2]^{+•}$	$[(M+2)-2Cl]^{+•}$
307.8958	$C_{12}H_4O^{35}Cl_2^{37}Cl_2$	$[M+4]^{+•}$	$[(M+4)-2Cl]^{+•}$
315.9419	$^{13}C_{12}H_4O^{35}Cl_4$	$[M]^{+•}$	-
317.9389	$^{13}C_{12}H_4O^{35}Cl_3^{37}Cl_1$	$[M+2]^{+•}$	-
373.8393	$C_{12}H_4O^{35}Cl_6$	-	$[M]^{+•}$
375.8364	$C_{12}H_4O^{35}Cl_5^{37}Cl_1$	-	$[M+2]^{+•}$
377.8334	$C_{12}H_4O^{35}Cl_4^{37}Cl_2$	-	$[M+4]^{+•}$
354.9792	-----	PFK lock mass	-----
Descriptor 2 Accurate mass	Elemental composition	Ion ID	
		PeCDF	HpCDFE
276.8959	$C_{11}H_3^{35}Cl_3^{37}Cl_1$	$[(M+2)-COCl]^+$	$[(M+2)-2Cl-COCl]^+$
337.8626	$C_{12}H_3O^{35}Cl_5$	$[M]^{+•}$	$[M-2Cl]^{+•}$
339.8597	$C_{12}H_3O^{35}Cl_4^{37}Cl_1$	$[M+2]^{+•}$	$[(M+2)-2Cl]^{+•}$
341.8567	$C_{12}H_3O^{35}Cl_3^{37}Cl_2$	$[M+4]^{+•}$	$[(M+4)-2Cl]^{+•}$
351.9000	$^{13}C_{12}H_3O^{35}Cl_4^{37}Cl_1$	$[M+2]^{+•}$	-
353.8970	$^{13}C_{12}H_3O^{35}Cl_3^{37}Cl_2$	$[M+4]^{+•}$	-
407.8004	$C_{12}H_3O^{35}Cl_7$	-	$[M]^{+•}$
409.7974	$C_{12}H_3O^{35}Cl_6^{37}Cl_1$	-	$[M+2]^{+•}$
411.7945	$C_{12}H_3O^{35}Cl_5^{37}Cl_2$	-	$[M+4]^{+•}$
354.9792	-----	PFK lock mass	-----
Descriptor 3 Accurate mass	Elemental composition	Ion ID	
		HxCDF	OCDFE
310.8570	$C_{11}H_2^{35}Cl_4^{37}Cl_1$	$[(M+2)-COCl]^+$	$[(M+2)-2Cl-COCl]^+$
371.8238	$C_{12}H_2O^{35}Cl_6$	$[M]^{+•}$	$[M-2Cl]^{+•}$
373.8208	$C_{12}H_2O^{35}Cl_5^{37}Cl_1$	$[M+2]^{+•}$	$[(M+2)-2Cl]^{+•}$
375.8178	$C_{12}H_2O^{35}Cl_4^{37}Cl_2$	$[M+4]^{+•}$	$[(M+4)-2Cl]^{+•}$
383.8642	$^{13}C_{12}H_2O^{35}Cl_6$	$[M]^{+•}$	-
385.8610	$^{13}C_{12}H_2O^{35}Cl_5^{37}Cl_1$	$[M+2]^{+•}$	-
443.7584	$C_{12}H_2O^{35}Cl_7^{37}Cl_1$	-	$[M+2]^{+•}$
445.7555	$C_{12}H_2O^{35}Cl_6^{37}Cl_2$	-	$[M+4]^{+•}$
447.7525	$C_{12}H_2O^{35}Cl_5^{37}Cl_3$	-	$[M+6]^{+•}$
380.9760	-----	PFK lock mass	-----

Table 5 (continued)

Descriptor 4 Accurate mass	Elemental composition	Ion ID	
		HpCDF	NCDPE
344.8180	C ₁₁ H ₃₅ Cl ₅ ³⁷ Cl ₁	[(M+2)-COCl] ⁺	[(M+2)-2Cl-COCl] ⁺
407.7818	C ₁₂ H ₁₀ ³⁵ Cl ₆ ³⁷ Cl ₁	[M+2] ⁺	[(M+2)-2Cl] ⁺
409.7789	C ₁₂ H ₁₀ ³⁵ Cl ₅ ³⁷ Cl ₂	[M+4] ⁺	[(M+4)-2Cl] ⁺
411.7760	C ₁₂ H ₁₀ ³⁵ Cl ₄ ³⁷ Cl ₃	[M+6] ⁺	[(M+6)-2Cl] ⁺
417.8253	¹³ C ₁₂ H ₁₀ ³⁵ Cl ₇	[M] ⁺	-
419.8220	¹³ C ₁₂ H ₁₀ ³⁵ Cl ₆ ³⁷ Cl ₁	[M+2] ⁺	-
477.7195	C ₁₂ H ₁₀ ³⁵ Cl ₈ ³⁷ Cl ₁	-	[M+2] ⁺
479.7165	C ₁₂ H ₁₀ ³⁵ Cl ₇ ³⁷ Cl ₂	-	[M+4] ⁺
481.7136	C ₁₂ H ₁₀ ³⁵ Cl ₆ ³⁷ Cl ₃	-	[M+6] ⁺
430.9728	-----	PFK lock mass	-----

Descriptor 5 Accurate mass	Elemental composition	Ion ID	
		OCDF	DCDPE
378.7790		[(M+2)-COCl] ⁺	[(M+2)-2Cl-COCl] ⁺
441.7428	C ₁₂ O ³⁵ Cl ₇ ³⁷ Cl ₁	[M+2] ⁺	[(M+2)-2Cl] ⁺
443.7399	C ₁₂ O ³⁵ Cl ₆ ³⁷ Cl ₂	[M+4] ⁺	[(M+4)-2Cl] ⁺
445.7370	C ₁₂ O ³⁵ Cl ₅ ³⁷ Cl ₃	[M+6] ⁺	[(M+6)-2Cl] ⁺
511.6805	C ₁₂ O ³⁵ Cl ₉ ³⁷ Cl ₁	-	[M+2] ⁺
513.6775	C ₁₂ O ³⁵ Cl ₈ ³⁷ Cl ₂	-	[M+4] ⁺
515.6746	C ₁₂ O ³⁵ Cl ₇ ³⁷ Cl ₃	-	[M+6] ⁺
454.9728	-----	PFK lock mass	-----

Table 6. Polychlorinated Diphenylethers (PCDPE)
Calibration Standards^a (pg/ μ L)

	STD 1	STD 2	STD 3	STD 4
3,3',4,4'-TCDF	32	320	640	1,600
2,2',4,4',5-PCDF	32	320	640	1,600
2,2',3,3',4,4'-HxCDF	32	320	640	1,600
2,2',4,4',5,5'-HxCDF ^b	32	320	640	1,600
2,2',3,4',5,5',6-HpCDF ^b	32	320	640	1,600
2,2',3',4,4',5,5',6'-OCDF ^b	32	320	640	1,600
2,2',3,3',4,4',5,5',6'-NCDF ^b	32	320	640	1,600
2,2',3,3',4,4',5,5',6,6'-DCDF ^b	32	320	640	1,600
<u>Internal Quantitation Standard</u>				
¹³ C ₁₂ -2,3,7,8-TCDF	50	50	50	50
¹³ C ₁₂ -1,2,3,7,8-PeCDF	50	50	50	50
¹³ C ₁₂ -1,2,3,4,7,8-HxCDF	125	125	125	125
¹³ C ₁₂ -1,2,3,4,6,7,8-HpCDF	125	125	125	125
<u>Recovery Standard</u>				
¹³ C ₁₂ -1,2,3,4-TCDD	50	50	50	50
¹³ C ₁₂ -1,2,3,4,7,8-HxCDD	125	125	125	125

^aStandard solution of 1,600 pg/ μ L of each PCDPE was provided by Dr. D. T. Williams of Health and Welfare Canada. All other standards were available through Cambridge Isotope Laboratories, Woburn, Massachusetts.

^bReference standards used to establish RRF factors for PCDPEs. The RRF values for HxCDF were calculated vs. ¹³C₁₂-TCDF, HpCDF vs. ¹³C₁₂-PeCDF, OCDF vs. ¹³C₁₂-HxCDF, and NCDF and DCDF vs. ¹³C₁₂-HpCDF.

RRFs were established for the available PCDFs versus the ^{13}C -PCDF internal quantitation standard (IQS) by analyzing four standards ranging from 32 to 1,600 pg/ μL for each available isomer. The chlorinated IQS and recovery standard (RS) compounds were kept at the level of 50 to 125 pg/ μL , which was consistent with the levels previously added to the human adipose tissue samples.

C. Detection of Polybrominated Diphenylethers (PBDPEs) by HRGC/HRMS-SIM

Detection of the brominated diphenylether species was accomplished by adding ions characteristic of the PBDPE molecular ion cluster to the quantitation list and deleting the ions usually monitored for the PBDDs. The monitoring strategy was essentially the same as that described for the SIM confirmation of PCDF responses, except that three injections of each extract were required to determine the hexa- through decabrominated diphenylethers due to the wide mass range required for the polybrominated species. The hexa- and hepta- congeners were determined in the first injection; the octa- and nona- congeners in the second injection; and the decabromodiphenylether, DBDPE, in the third injection. Tris(perfluoroheptyl)-S-triazine was used to calibrate the mass scale for the determination of the DPDPPE because the abundance of the PFK ions above m/z 900 are very weak. The ions monitored for these analyses are given in Table 7.

Quantitation of the PBDPEs except the DPDPPE was not possible because only mixed isomer standards of brominated fire retardants (Bromkal 70-5-DE and 79-8-DE, and decabromodiphenylethers, Ultra Scientific) were commercially available. The Bromkal 70-5-DE has been reported to be comprised of 41.7% 2,2',4,4'-TBDPE, 44.4% 2,2',4,4',5'-PeBDPE, 7.6% other PeBDPE isomers, and 6% HxBDPE isomers.^{6,11} The composition of Bromkal 79-8-DE has been determined to contain 8% of HpDPE, 26% OBDPE, 46% NBDPE, and 19% of DBDPE, although no specific isomer designation have been reported.⁶ These standards were used to establish approximate retention time windows and ion ratios for the PBDPE homolog series. A commercial standard of DBDPE was prepared and used to establish the retention time and spectra for that compound. This standard was used as an external standard (240 pg/ μL) for comparison of responses to those measured in the sample extracts.

Table 7. Ions Monitored for the HRGC/HRMS-SIM Determination of PBDPE

<u>HxBDPE</u>		Ion ID	
Accurate mass	Elemental composition	TBDF	HxBDPE
374.7846	$C_{11}H_4^{79}Br_2^{81}Br_1$	$[(M+2)-COBr]^+$	$[(M+2)-2Br-COBr]^+$
481.6978	$C_{12}H_4O^{79}Br_3^{81}Br_1$	$[M+2]^+$	$[(M+2)-2Br]^+$
483.6959	$C_{12}H_4O^{79}Br_2^{81}Br_2$	$[M+4]^+$	$[(M+4)-2Br]^+$
485.6939	$C_{12}H_4O^{79}Br_1^{81}Br_3$	$[M+6]^+$	$[(M+6)-2Br]^+$
493.7381	$^{13}C_{12}H_4O^{79}Br_3^{81}Br_1$	$[M+2]^+$	-
495.7362	$^{13}C_{12}H_4O^{79}Br_2^{81}Br_2$	$[M+4]^+$	-
641.5326	$C_{12}H_4O^{79}Br_4^{81}Br_2$	-	$[M+4]^+$
643.5306	$C_{12}H_4O^{79}Br_3^{81}Br_3$	-	$[M+6]^+$
645.5287	$C_{12}H_4O^{79}Br_2^{81}Br_4$	-	$[M+8]^+$
480.9697	-----	PFK lock mass	-----

<u>HpBDPE</u>		Ion ID	
Accurate mass	Elemental composition	PeBDF	HpBDPE
454.6931	$C_{11}H_3^{79}Br_2^{81}Br_1$	$[(M+4)-COBr]^+$	$[(M+4)-2Br-COBr]^+$
559.6084	$C_{12}H_3O^{79}Br_4^{81}Br_1$	$[M+2]^+$	$[(M+2)-2Br]^+$
561.6064	$C_{12}H_3O^{79}Br_3^{81}Br_2$	$[M+4]^+$	$[(M+4)-2Br]^+$
563.6044	$C_{12}H_3O^{79}Br_2^{81}Br_3$	$[M+6]^+$	$[(M+6)-2Br]^+$
573.6466	$^{13}C_{12}H_3O^{79}Br_3^{81}Br_2$	$[M+4]^+$	-
575.6447	$^{13}C_{12}H_3O^{79}Br_2^{81}Br_3$	$[M+6]^+$	-
719.4432	$C_{12}H_3O^{79}Br_5^{81}Br_2$	-	$[M+4]^+$
721.4412	$C_{12}H_3O^{79}Br_4^{81}Br_3$	-	$[M+6]^+$
723.4392	$C_{12}H_3O^{79}Br_3^{81}Br_4$	-	$[M+8]^+$
580.9633	-----	PFK lock mass	-----

<u>OBDPE</u>		Ion ID	
Accurate mass	Elemental composition	HxBDF	OBDPE
532.6036	$C_{11}H_2^{79}Br_3^{81}Br_2$	$[(M+4)-COBr]^+$	$[(M+4)-2Br-COBr]^+$
639.5169	$C_{12}H_2O^{79}Br_4^{81}Br_2$	$[M+4]^+$	$[(M+4)-2Br]^+$
641.5150	$C_{12}H_2O^{79}Br_3^{81}Br_3$	$[M+6]^+$	$[(M+6)-2Br]^+$
643.5130	$C_{12}H_2O^{79}Br_2^{81}Br_4$	$[M+8]^+$	$[(M+8)-2Br]^+$
799.3518	$C_{12}H_2O^{79}Br_5^{81}Br_3$	-	$[M+6]^+$
801.3498	$C_{12}H_2O^{79}Br_4^{81}Br_4$	-	$[M+8]^+$
803.3478	$C_{12}H_2O^{79}Br_3^{81}Br_5$	-	$[M+10]^+$
580.9633	-----	PFK lock mass	-----

Table 7 (continued)

<u>NBDPE</u>			
Accurate mass	Elemental composition	Ion ID	
		HpBDF	NBDPE
612.5120	C ₁₁ H ⁷⁹ Br ₃ ⁸¹ Br ₂	[(M+6)-COBr] ⁺	[(M+6)-2Br-COBr] ⁺
719.4250	C ₁₂ HO ⁷⁹ Br ₄ ⁸¹ Br ₃	[M+6] ^{+•}	[(M+6)-2Br] ^{+•}
723.4210	C ₁₂ HO ⁷⁹ Br ₂ ⁸¹ Br ₅	[M+10] ^{+•}	[(M+10)-2Br] ^{+•}
877.2623	C ₁₂ HO ⁷⁹ Br ₆ ⁸¹ Br ₃	-	[M+6] ^{+•}
879.2604	C ₁₂ HO ⁷⁹ Br ₅ ⁸¹ Br ₄	-	[M+8] ^{+•}
881.2584	C ₁₂ HO ⁷⁹ Br ₄ ⁸¹ Br ₅	-	[M+10] ^{+•}
580.9633	-----	PFK lock mass	-----
<u>DBDPE</u>			
Accurate mass	Elemental composition	Ion ID	
		OBDF	DBDPE
957.1709	C ₁₂ O ⁷⁹ Br ₆ ⁸¹ Br ₄	-	[M+8] ^{+•}
959.1690	C ₁₂ O ⁷⁹ Br ₅ ⁸¹ Br ₅	-	[M+10] ^{+•}
961.1670	C ₁₂ O ⁷⁹ Br ₄ ⁸¹ Br ₆	-	[M+12] ^{+•}
915.9550	--- tris-(perfluoroheptyl)-5-triazine lock mass ---		

V. RESULTS

The results of the confirmation analysis efforts are presented in this section. The supporting data include summaries of calibration efforts, estimates of concentrations for isomers, and identification of specific chlorinated diphenylether isomers and related concentrations. Chromatographic data from the HRGC/ HRMS-SIM and full scan experiments are presented to support the confirmation of the presence of PCDEs and PBDEs along with the measured versus theoretical ion ratios for the molecular clusters.

A. Full Scan HRGC/MS Confirmation of PCDEs and PBDEs

Full scan HRGC/MS analysis of selected adipose extracts confirmed the presence of a hexabromodiphenylether, HxBDE, and a nonachlorodiphenylether, NCDPE. These congeners were estimated to be at the highest concentrations in each of the adipose extracts. Figure 1 presents the full scan HRGC/MS chromatogram for sample 16289 (ACD8700167). Figures 2 and 3 show representative mass spectra of the NCDPE and HxBDE detected in the standards and samples. The spectra of NCDPE is compared to an authentic standard in Figure 2. A reference compound was not available to compare the spectrum of HxBDE. However, the fragmentation pattern is comparable with reference spectra from the literature.^{6,7,9}

Molecular (M^+) and fragment ions in the electron impact (EI) mass spectra of these polyhalogenated compounds show the expected clustering due to the chlorine isotopes, ^{35}Cl and ^{37}Cl , and the bromine isotopes, ^{79}Br and ^{81}Br . The EI mass spectra of the nonachloro- and hexabromo DPEs are representative of the fragmentation patterns as observed for these groups of compounds. All PHDPEs have very strong molecular [M^+] ions and major (often base peaks) fragment ions ($M^+-\text{Cl}_2$ or $M^+-\text{Br}_2$). These latter ions have the same exact mass and number of halogen ions as the corresponding PHDFs, which give rise to the potential for false positive determination of PHDFs in the presence of the halogenated DPEs.

Further fragment ions characteristic (and relative response) of the polyhalogenated DPEs include: $M^+-\text{Cl}$ (weak), $M^+-\text{COCl}$ (median/weak), $M^+-\text{Cl}_2$ (weak), $M^+-\text{Cl}_2-\text{COCl}$ (medium), and $M^+-\text{Cl}_4$ (medium): Due to the cluster formation from the chlorine isotopes the most prominent ions in these clusters may be at higher m/z values as evidenced for the NCDPE (Figure 2).⁸

In comparison to the chlorinated DPEs, the EI mass spectra for PCDFs exhibit M^+ ions that are the base peaks in the spectrum. Other fragment ions which are characteristic of the PCDFs are: $M^+-\text{Cl}$ (weak), $M^+-\text{COCl}$ (medium/weak), $M^+-\text{Cl}$ (weak/medium), and $M^+-\text{COCl}-\text{Cl}_2$ (medium). A key distinction between the spectrum of the PCDFs and the PCDEs is that the base peak for PCDFs is typically at the M^+ cluster while the base peak for the PCDEs is at the $M^+-\text{Cl}_2$ cluster.⁸

Similar fragmentation patterns are characteristic of the brominated DPEs and the PBDFs. As for the chlorinated compounds the base peak in the PBDF spectra can be found in the M^+ cluster, while the PBDE exhibits the base peak at the $M^+-\text{Br}_2$ cluster and a strong response at the M^+ cluster.^{6,9}

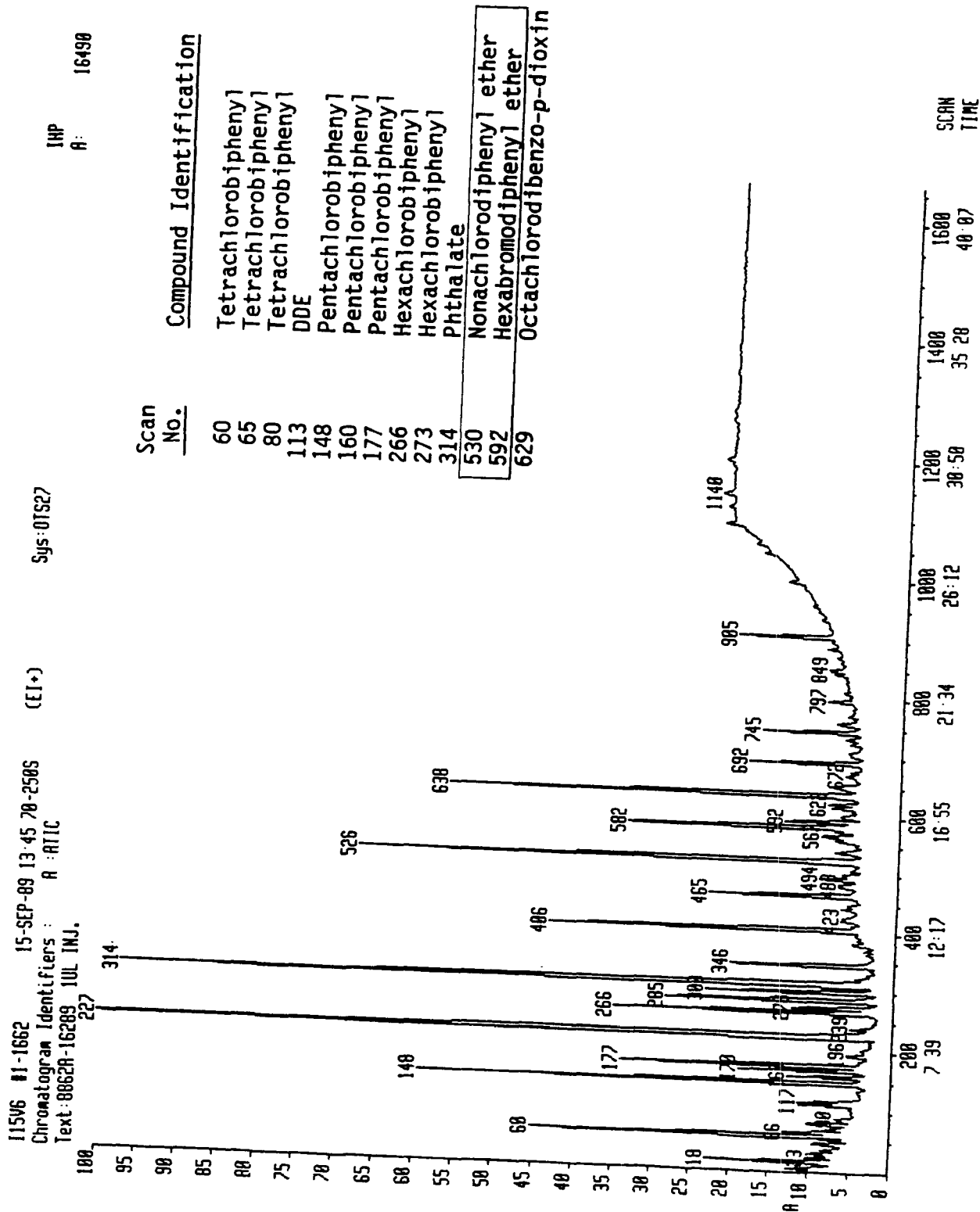


Figure 1. Reconstructed ion chromatogram from the HRGC/MS full scan (100-1000 amu) analysis of sample 16289 (ACD8700167).

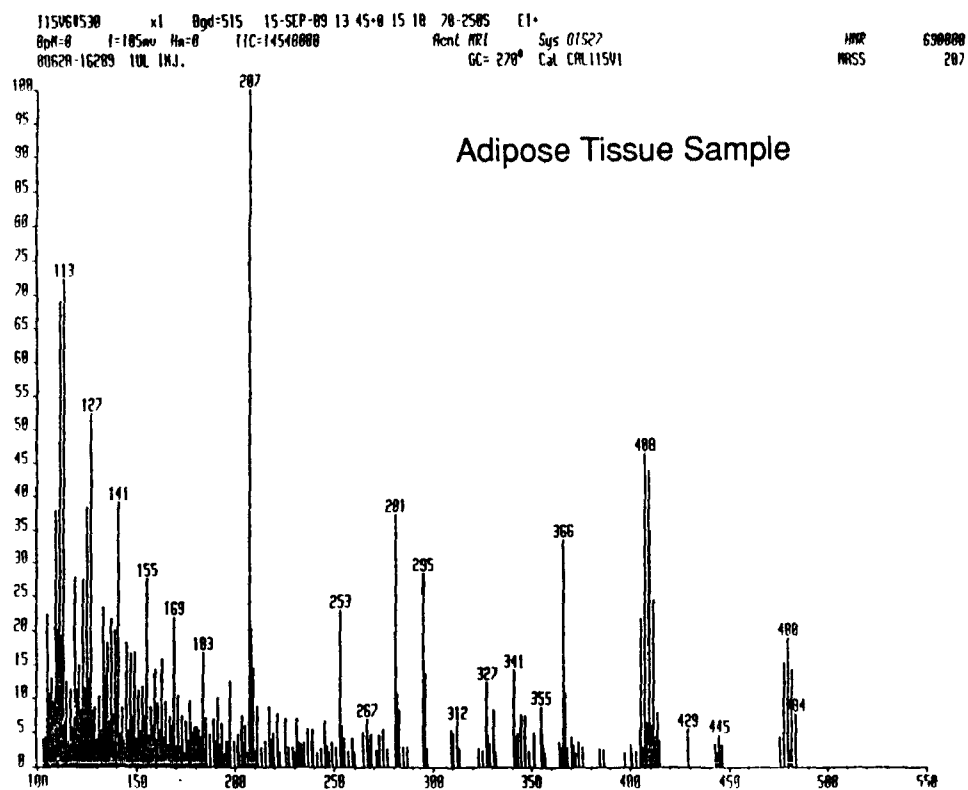
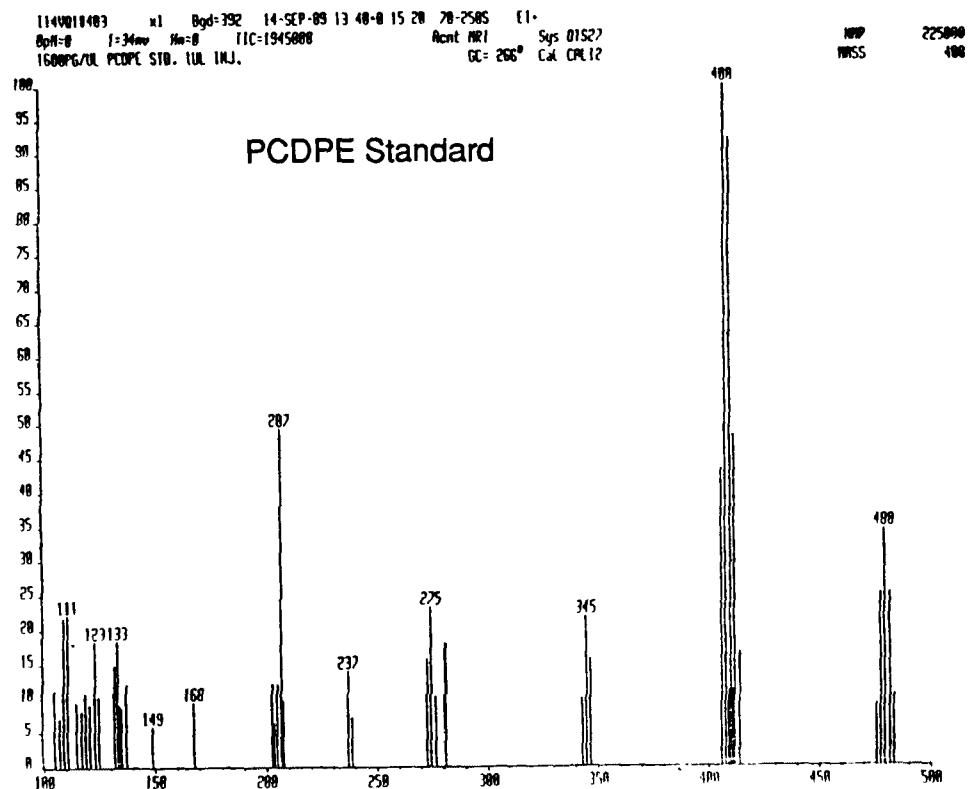


Figure 2. Comparison of the full scan (100-1000 amu) mass spectra of a 2,2',3,3',4,4',5,5',6'-NCDPE standard vs. a NCDPE isomer response in sample 16289 (ACD8700167).

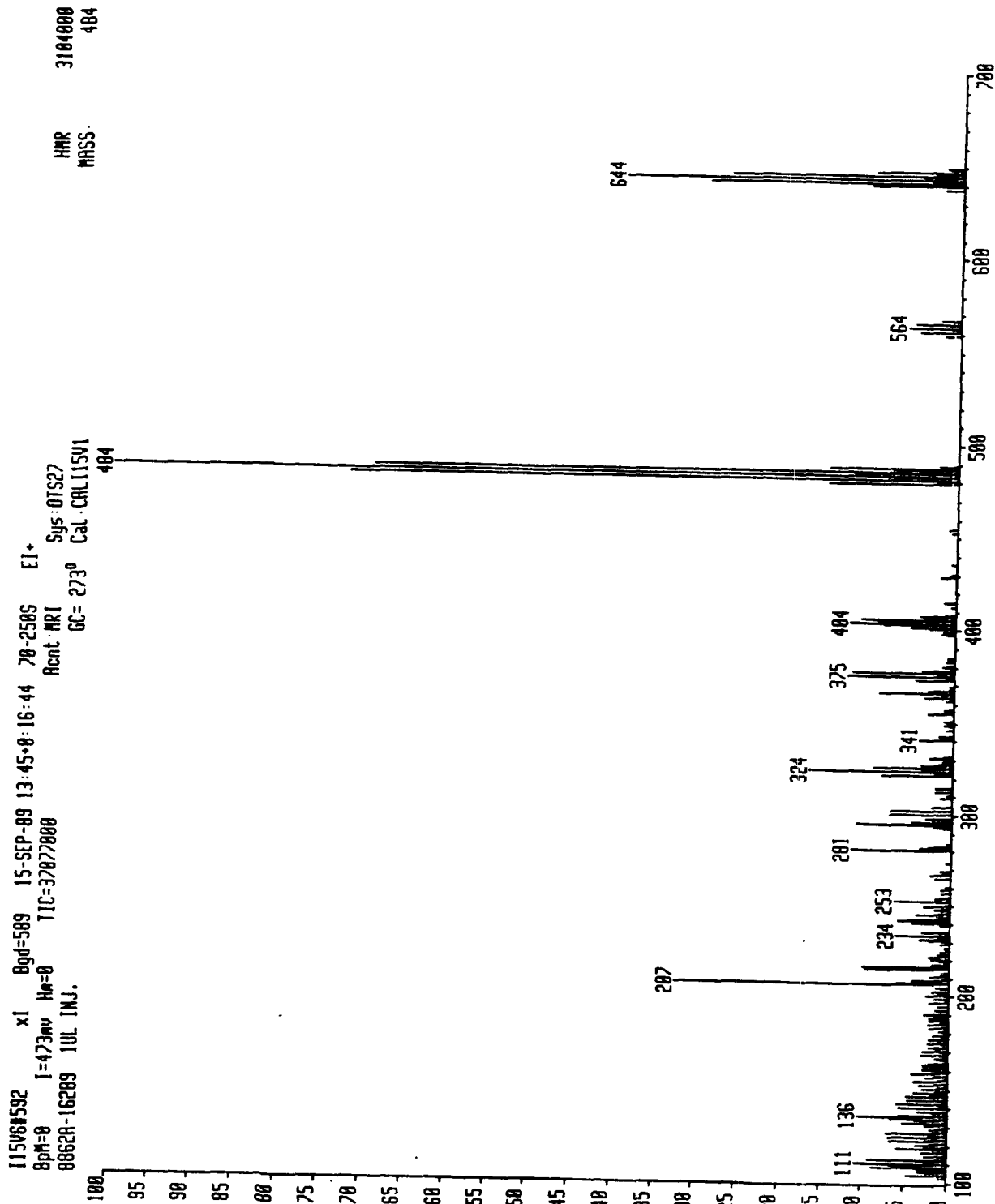


Figure 3. Full scan (100-1000 amu) mass spectra of a Hx8DPE isomer in sample 16289 (ACD8700167).

In addition to the bromo- and chlorodiphenylethers, the full scan HRGC/MS analysis identified the presence of eight PCB congeners. Specifically, tetrachlorobiphenyls (3 isomers), pentachlorobiphenyls (3 isomers), and hexachlorobiphenyls (2 isomers) were detected and compared to a reference library for identification. Since these isomers were recovered from the final cleanup column of the sample preparation for these samples (AX-21 on silica gel), it is likely that these isomers are co-planar (nonortho-substituted). OCDD was also confirmed in the extracts by full scan mass spectrometry. However, the other PCDDs and PCDFs were not detected due to the low parts-per-trillion levels, which required SIM analyses.

B. HRGC/HRMS-SIM Confirmation/Quantitation of Polychlorinated DPES (PCDPES)

The HRGC/HRMS-SIM analyses for polychlorinated DPES required establishing a calibration curve using the available PCDPPE congeners. The ions characteristic of the loss of two chlorine atoms from the PCDPPE molecular cluster (which are consistent with the characteristic ions of the PCDF molecular clusters) plus two ions characteristic of the PCDF IQS (typically the $[M]^+$ and $[M+2]^+$ ions of the molecular cluster) were used to calculate the RRF values:

- The RRF for HxCDPE was calculated versus ^{13}C -TCDF
- The RRF for HpCDPE was calculated versus ^{13}C -PeCDF
- The RRF for OCDPE was calculated versus ^{13}C -HxCDF
- The RRFs for NCDPE and DCDPE were calculated versus the ^{13}C -HpCDF

The RRFs of these PCDPES versus the ^{13}C -PCDF IQS are given in Table 8. Relative standard deviations of the RRFs over the 50-fold range in concentration were less than 20%.

The interpretation of the mass chromatograms from the HRGC/HRMS-SIM analysis was based on the comparison of observed ratios between characteristic ions and the predicted or theoretical ratios calculated on isotopic abundance. Table 9 is a summary of the ion ratios measured from the mass chromatograms for each sample. This table presents six combinations based on ion ratios within the molecular clusters and at the masses representing loss of two chlorines from the molecular clusters.

Comparison of characteristic ion ratios between the molecular clusters and the ion clusters from the loss of two chlorines was not possible since this would have required establishing the ratios versus authentic isomers. The number of authentic standards available was not sufficient to establish these relationships, since the ratios of the molecular clusters versus the fragment clusters are expected to vary for specific isomers. However, the fragment characteristic of the $M^+-\text{Cl}_2$ cluster were observed to be higher in response than the ions characteristic of the molecular, M^+ cluster. This is representative of the spectra of the PCDPES. PCDFs in contrast exhibit the greatest response at the M^+ cluster.

Table 8. Chlorinated Diphenylether Relative Response Factors (RRFs)

COMPOUND	Masses	Retention Time (min)	Relative Response Factor				Mean RRF	%RSD
			32 pg/ μ l	320 pg/ μ l	640 pg/ μ l	1600 pg/ μ l		
2,2',4,4',5,5'-HxCdPE	304/306	28:38	0.830	1.034	1.221	1.326	1.103	19.8
2,2',3,4',5,5',6-HpCDPE	340/342	34:50	0.893	0.845	0.983	1.142	0.966	13.5
2,2',3',4',4',5,5',6'-OCDPE	374/376	41:51	1.084	1.035	1.135	1.291	1.136	9.8
2,2',3,3',4,4',5,5',6'-NCDPE	408/410	47:00	1.366	1.214	1.371	1.477	1.357	8.0
2,2',3,3',4,4',5,5',6,6'-DCDPE	442/444	49:55	1.076	1.076	1.122	1.119	1.098	2.3

Table 9. Theoretical Vs. Measured Ion Ratios for PCDPs in the FY87 NHATS Samples

No. of Cl	Characteristic ions	Theoretical ion ratio	Sample 16313 (ACD8700452)	Sample 16279 (ACD8700185)	Sample 16289 (ACD8700167)	Sample 16269 (ACD8700283)	Sample 16286 (ACD8700381)
Hexa (6)	304/306	0.761	0.609	0.685	0.606 ^a	0.643	0.586 ^a
	306/308	2.018	2.166	2.029	2.429 ^a	2.188	2.221
	304/308	1.535	1.321	1.389	1.214 ^a	1.408	1.300
	374/376	0.508	0.541	0.497	0.460	0.539	0.544
	376/378	1.217	1.299	1.202	1.382	1.244	1.370
	374/378	0.619	0.703	0.597	0.636	0.670	0.745
Hepta (7)	338/340	0.609	0.566	0.604	0.560	0.609	0.622
	340/342	1.519	1.641	1.469	1.580	1.549	1.423
	338/342	0.925	0.929	0.888	0.928	0.944	0.884
	408/410	0.436	0.476	0.518	0.417	0.446	b
	410/412	1.016	0.991	0.924	1.186	1.292 ^a	1.250 ^a
	408/412	0.443	0.472	0.479	0.539 ^a	0.474	b
Octa (8)	372/374	0.508	0.473	0.499	0.484	0.499	0.498
	374/376	1.217	1.363	1.434	1.348	1.260	1.266
	372/376	0.619	0.645	0.662	0.661	0.630	0.631
	444/446	0.872	0.839	0.807	0.889	0.835	0.818
	446/448	1.523	1.647	1.534	1.654	1.674	1.508
	444/448	1.327	1.382	1.238	1.470	1.353	1.350
Nona (9)	408/410	1.016	1.063	1.034	0.992	0.974	1.082
	410/412	1.825	1.962	1.822	1.887	1.909	1.951
	408/412	1.854	2.085	1.883	1.872	1.859	2.111
	478/480	0.763	0.791	0.808	0.754	0.764	0.787
	480/482	1.307	1.386	1.357	1.457	1.365	1.395
	478/482	0.997	1.097	1.097	1.098	1.043	1.098
Deca (10)	442/444	0.872	0.827	0.823	0.859	0.828	0.797
	444/446	1.523	1.617	1.589	1.493	1.597	1.591
	442/446	1.327	1.336	1.307	1.282	1.323	1.268
	512/514	0.679	0.719	0.644	0.645	0.658	0.553
	514/516	1.144	1.202	1.151	1.173	1.187	1.236
	512/516	0.776	0.719	0.743	0.757	0.781	0.683

^a ion ratio outside of $\pm 20\%$ window for the theoretical ratio.
^b ion at m/z 408 not measured due to overlapping interference.

The presence of the fragments representing the combined losses of two chlorines and COCl from the molecular cluster were also used to confirm the presence of the PCDPes. For some PCDPe homologs, particularly the OCDPe, more than one chromatographic response was noted in the analysis of each extract, indicating the presence of more than one isomer. These data demonstrated that a consistent pattern of PCDPes was detected in each sample.

The confirmation efforts included analysis of a laboratory method blank (Lab no. 16278). This sample was prepared along with the adipose tissue and consisted of all reagents taken through each of the sample preparation steps. This sample did not exhibit any responses to the PCDPes, providing evidence that there was no laboratory background contribution to the samples.

The quantitative results for the five samples analyzed under these conditions are presented in Table 10 and compared with estimated results reported in Table 1. The data in Table 10 are presented for each response quantitated in the confirmational analyses and are compared to the total homolog values estimated in the original PCDD and PCDF analysis effort (Table 1).

Retention times are given as a measure of reliability and reproducibility in the identification of the 2,2',4,4',5,5'-HxCdPe and 2,2',3',4,4',5,5',6'-OCDPe isomers. The retention time and relative retention time versus the internal quantitation standards measured for these isomers in the analytical standards correspond to responses measured in the samples. Although there were two HxCdPe isomers in the standards mix, only the 2,2',4,4',5,5'-substituted isomer eluted in the TCDF retention time window. The other HxCdPe isomer (2,2',3,3',4,4'-substituted) eluted in the PCDF retention time window after the HpCdPe isomer, and was not quantified due to its low response in the HpCdPe mass range. This elution pattern is consistent with that reported by Williams and LeBel.¹⁰ The assignment of isomer designations to the PCDPe response should be considered tentative since the number of possible PCDPe congeners is analogous with PCBs. A total of 209 unique PCDPe congeners are possible. The tentative assignments presented in this report are based on matching retention times and do not account for potentially overlapping isomers.

The results calculated versus the PCDPe standards are comparable (generally within a factor of two or less) with the results in Table 1, which are estimates based on RRF values for the corresponding PCDF RRF value. Chloro DPe levels ranged from 2.8 ppt HpCdPe to 1,400 ppt NCDPe. Based on this comparison of the results the values calculated versus the PCDF standards in the initial effort are good approximations of the PCDPe levels in the tissue samples. Users of the data in Table 1, however, must recognize the limitations of the data set, which are discussed below.

Although standards were used to quantitate the levels of PCDPes in the samples, the values should still be considered as estimates for three reasons. First, the purity of the standards used to quantitate the levels of PCDPes in these samples have not been verified.

Table 10. Estimated PCDE Concentrations in Selected FY87 NHATS Samples

Compound	Retention time (min)	Concentration (pg/g)	
		RRF-PCDPE ^a	RRF-PCDF ^b
Sample ACD8700283			
2,2',4,4',5,5'-HxDPE	28:41	13	10
HpCDPE	38:18	10	5
2,2',3',4,4',5,5',6'-OCDPE	41:54	40	
OCDPE	43:04	20	
OCDPE	44:20	170	200 ^c
NCDPE	47:01	780	1000
DCDPE	49:55	90	ND
Sample ACD8700185			
2,2',4,4',5,5'-HxDPE	28:39	7.5	5
HpCDPE	36:36	2.8	
HpCDPE	38:16	6.1	5 ^d
2,2',3',4,4',5,5',6'-OCDPE	41:51	34	
OCDPE	43:02	16	
OCDPE	44:19	100	200 ^c
NCDPE	46:59	560	1000
DCDPE	49:54	75	10
Sample ACD8700381			
2,2',4,4',5,5'-HxDPE	28:36	5.6	4
HpCDPE	38:15	5.0	3
2,2',3',4,4',5,5',6'-OCDPE	41:50	29	
OCDPE	43:00	16	
OCDPE	44:17	110	100 ^c
NCDPE	46:57	760	1000
DCDPE	49:52	92	ND
Sample ACD8700167			
2,2',4,4',5,5'-HxDPE	28:39	5.7	4
HpCDPE	38:16	6.6	7
2,2',3',4,4',5,5',6'-OCDPE	41:51	28	
OCDPE	43:02	15	
OCDPE	44:19	67	100 ^c
NCDPE	46:59	800	1000
DCDPE	49:54	73	ND
Sample ACD8700452			
2,2',4,4',5,5'-HxDPE	28:37	12	10
HpCDPE	38:16	7.8	10
2,2',3',4,4',5,5',6'-OCDPE	41:51	32	
OCDPE	43:02	26	
OCDPE	44:18	190	200 ^c
NCDPE	46:59	1400	2000
DCDPE	49:54	140	ND

^aValue estimated from PCDE relative response factor values (2 significant figures).

^bValue estimated from previous effort for determination of PCDEs using PCDF-relative response factor values (1 significant figure).

^cTotal of all OCDPE observed from previous analysis effort. The total of OCDPE values for the current effort are obtained by adding the three values given.

^dTotal of all HpCDPE responses from the previous analysis effort.

ND = not detected.

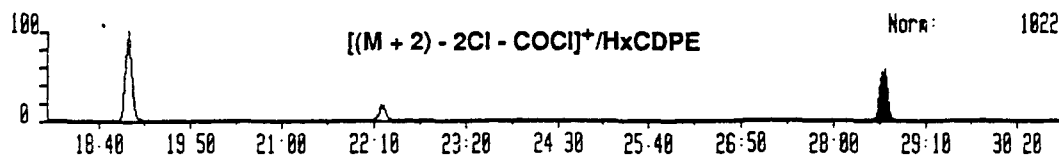
Second, the ions used for quantitation were those characteristic of the furan isomers, and as such, could contain contributions from actual furan isomers in addition to chloro DPE responses (this occurred particularly for the overlap of responses of two of the OCDPE isomers with the 1,2,3,6,7,8-HxCDF and the 2,3,4,6,7,8-HxCDF isomers). The potential contribution of the PCDFs could be further evaluated by establishing RRF value based on the molecular clusters of the PCDPE rather than the ions characteristic of the PCDF. The concentration based on the molecular clusters would provide a more accurate determination of the PCDPE.

Third, the recovery of the PCDPEs from the procedures used to generate the extracts is unknown. This point is of most concern, since using the PCDF IQS to calculate amounts in the samples it is assumed that the recovery of the PCDPEs is similar to the PCDFs. Hence the actual concentrations of the chloro DPEs may be higher than presented in this report. Previous attempts by other researchers to specifically determine the PCDPE levels in adipose tissues of the Canadian population were not successful in identifying these compounds. However, this study demonstrated that the PCDPEs did not exceed the 10 ng/g (ppb) level for a single response.¹⁰. Future studies for the determination of PCDPEs should incorporate the use of stable isotope-labeled PCDPEs to provide accurate determinations of PCDPEs.

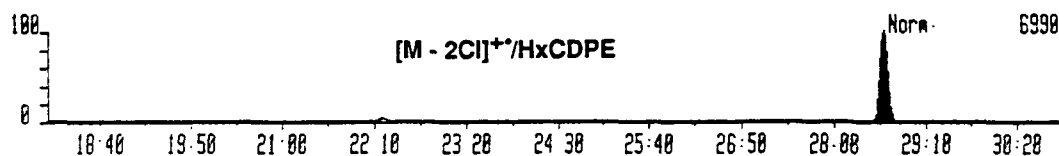
Figures 4 through 13 compare some of the characteristic ions of PCDPEs from the PCDPE standard and a representative sample. A consistent pattern of PCDPEs was observed in all of the sample extracts included in the confirmational analyses and in the original analysis effort for the PCDFs. Based on the responses observed in Figure 9, it is anticipated that there are additional isomers of OCDPE in the adipose tissue extract. This is based on the partially observed signal at 45 min for ions characteristic of OCDPE. Unfortunately, this response eluted during the switch from one set of mass descriptors for OCDPE to the descriptors for NCDPE.

Potential of PCDPE cyclization to form PCDFs--One of the concerns with the presence of high levels of PCDPEs in the sample extracts is the potential for cyclization in the injection port to form PCDDs or PCDFs resulting in false positive identification. This potential was evaluated by analyzing the highest available standard (1600 pg/ μ L) using the conditions normally used for PCDD and PCDF analysis. The results of this experiment did not provide evidence of this formation. In order to substantiate that the response observed as PCDPE did not originate due to sample handling, method blanks previously prepared with the sample were analyzed. The results of these analyses demonstrated that the laboratory was free of PCDPE background.

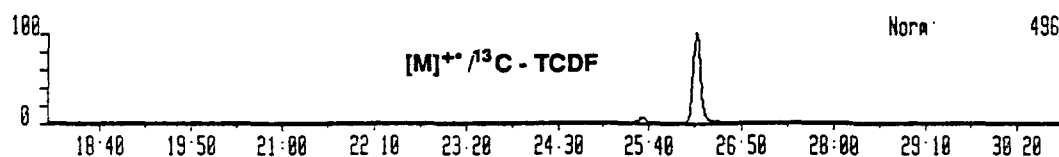
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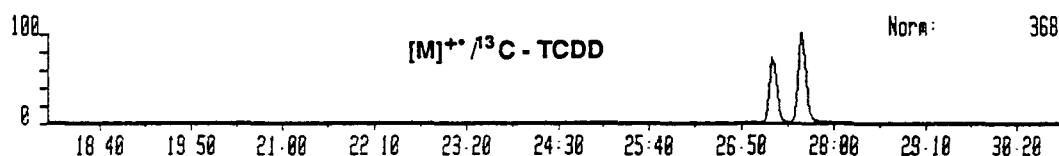
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111VQ5 11-SEP-89 Sir Voltage 70-250S Sys DIOXINS
 Sample 1 Injection 1 Group 1 Mass 315.9419
 Text:8862A-640PG/UL PCDFE STD. 1UL INJ.



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 Sample 1 Injection 1 Group 1 Mass 333.9339
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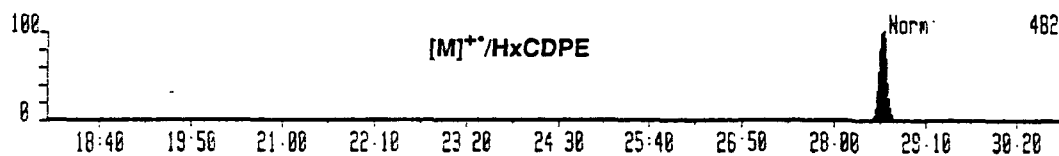
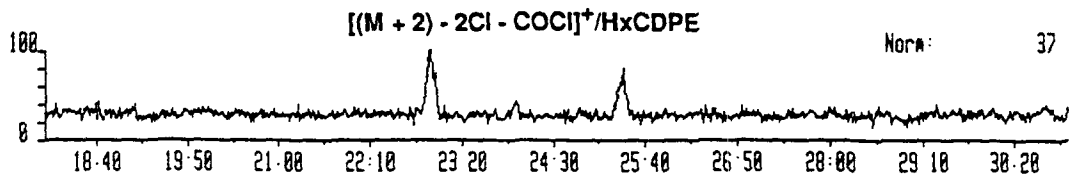
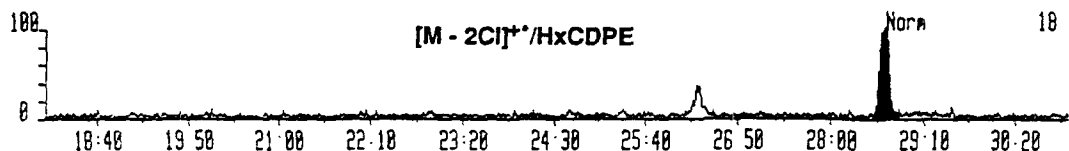


Figure 4. HRGC/HRMS-SIM mass chromatogram for determination of a 640-pg/ μ L standard of HxCDFE. The shaded peaks represent the response for 2,2',4,4',5,5'-HxCDFE. The $^{13}\text{C}_{12}$ -TCDF internal standard cluster at approximately 26:15.

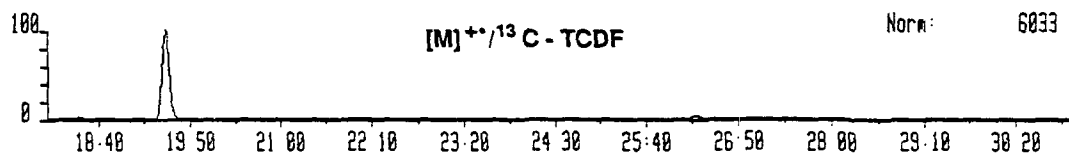
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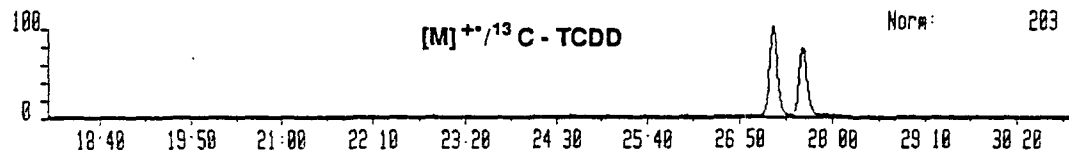
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112V2 12-SEP-89 Sir:Voltage 70-250S Sys: DIOXINS
 Sample 1 Injection 1 Group 1 Mass 333.9339
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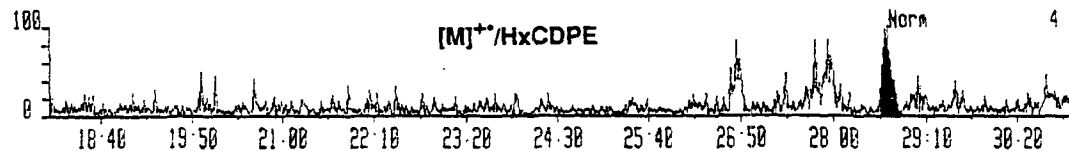
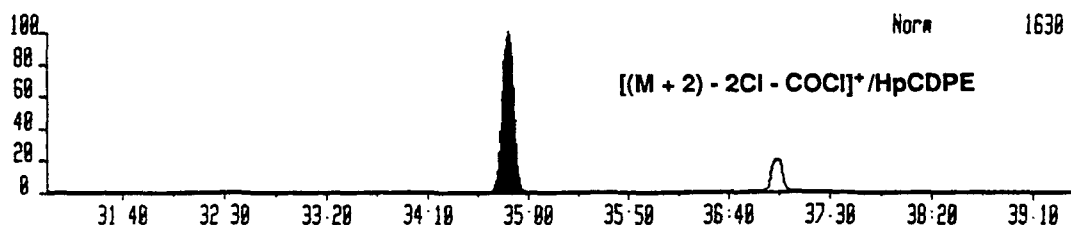
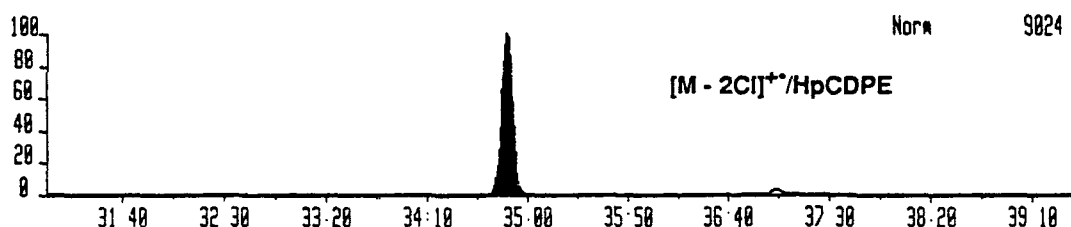


Figure 5. HRGC/HRMS-SIM mass chromatogram for the analysis of sample 16289 (ACD8700167) for HxCDF. The shaded peaks represent the response for a HxCDF isomer. The ¹³C₁₂-TCDF internal standard cluster at approximately 26:15.

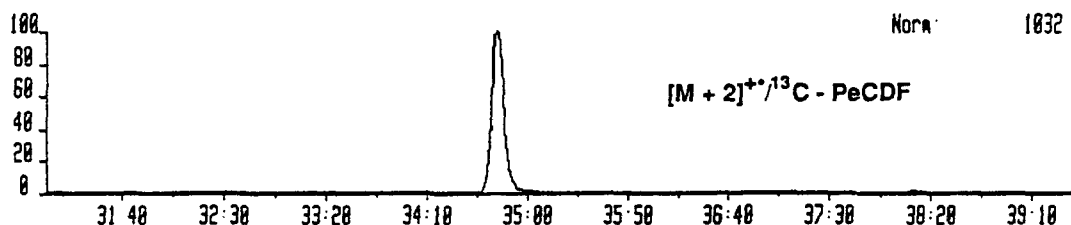
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 Sample 1 Injection 1 Group 2 Mass 337.8626
 Text 8862R-640PG/UL PCDFE STD. 1UL INJ.



111VQ5 11-SEP-89 Sir:Voltage 70-250S Sys: DIOXINS
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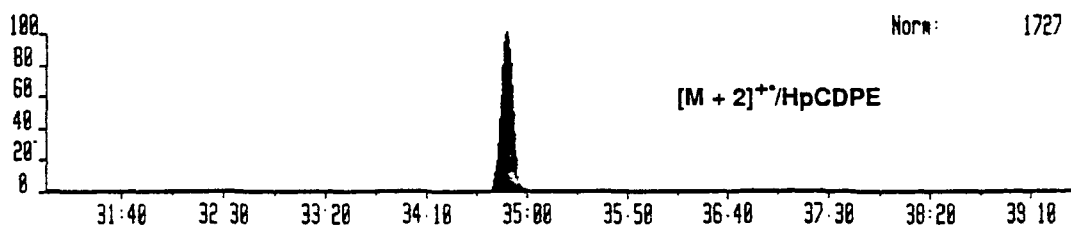
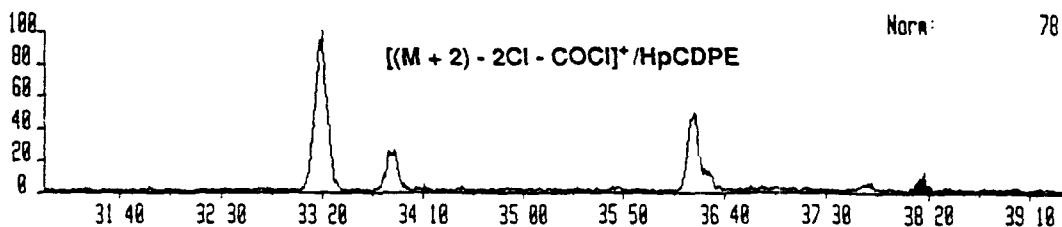
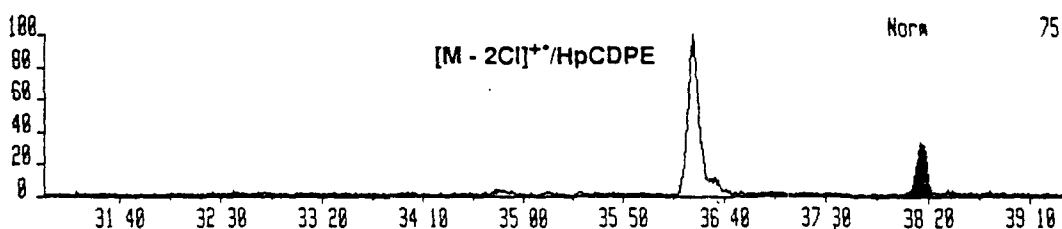


Figure 6. HRGC/HRMS-SIM mass chromatogram from the analysis of a 640-pg/ μL standard of 2,2',3,4',5,5',6'-HpCDPE. The shaded peaks represent the HpCDPE response.

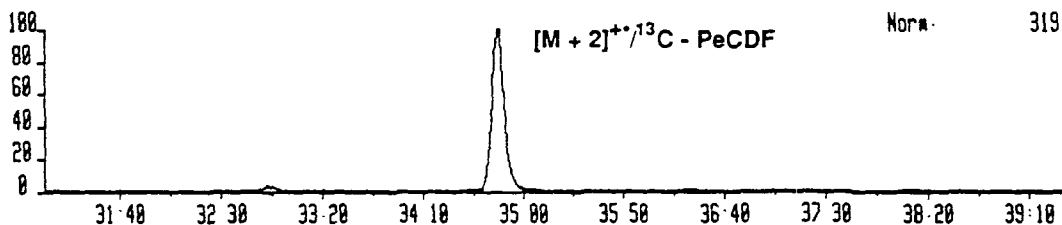
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 Sample 1 Injection 1 Group 2 Mass 337.8626
 Text 8862A-16289 1UL INJ.



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 Sample 1 Injection 1 Group 2 Mass 351.9808
 Text 8862A-16289 1UL INJ.



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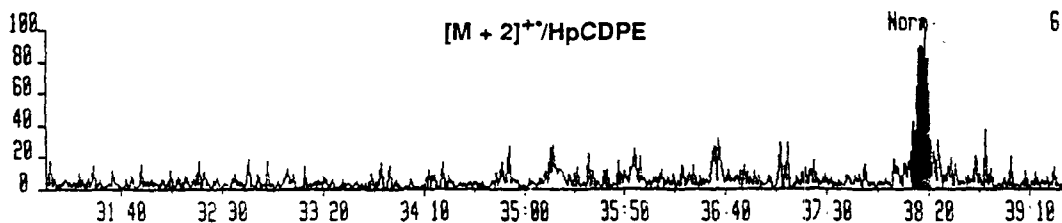
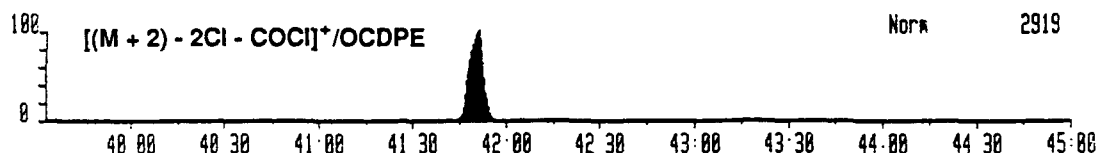
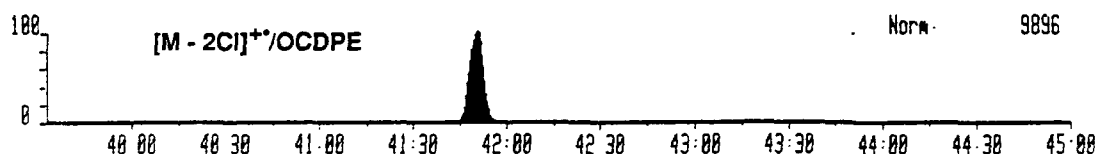


Figure 7. HRGC/HRMS-SIM mass chromatogram from the analysis of sample 16289 (ACD8700167) for HpCDPE. The shaded peaks represent the response for a HpCDPE isomer. The responses for m/z 277 and m/z 338 at 36:25 represent 2,3,4,7,8-PeCDF.

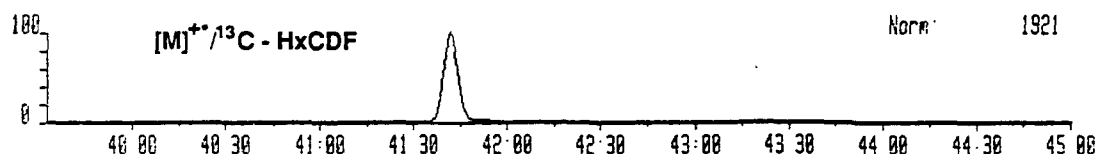
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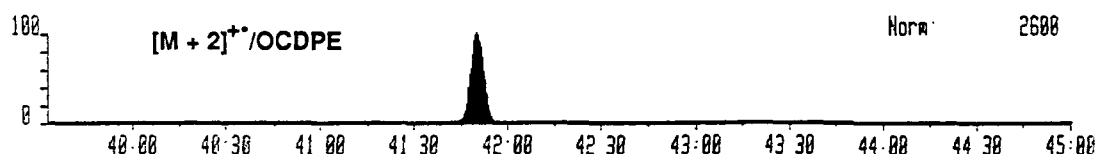
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 Sample 1 Injection 1 Group 3 Mass 383.8642
 Text 8862A-640PG/UL PCOPE STD. 1UL INJ.



111VQ5 11-SEP-89 Sur Voltage 70-250S Sys DIOXINS
 Sample 1 Injection 1 Group 3 Mass 443.7584
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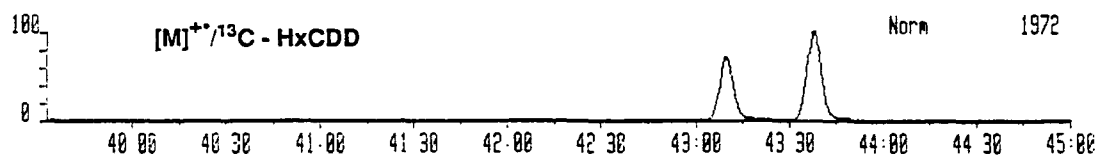
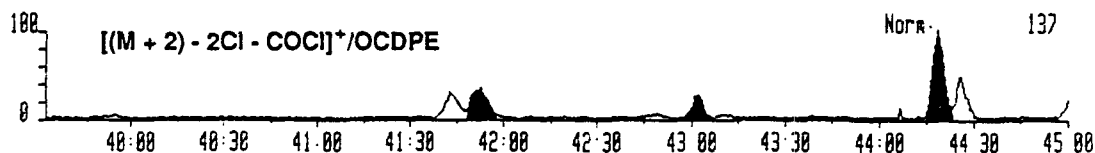
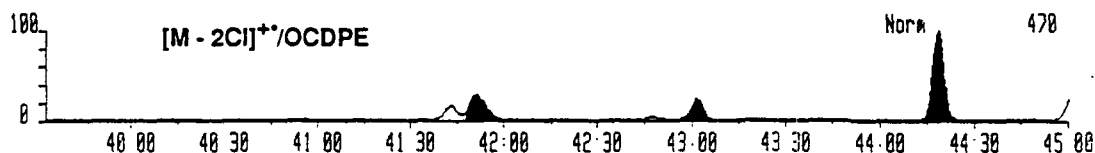


Figure 8. HRGC/HRMS-SIM mass chromatogram from the analysis of a 640-pg/ μ L standard of 2,2',3',4,4',5,5',6'-OCDPE. The shaded peaks represent the OCDPE response.

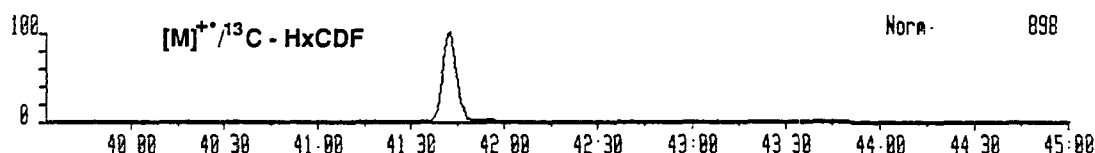
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 Sample 1 Injection 1 Group 3 Mass 310.8570
 Text 8862A-16289 1UL INJ.



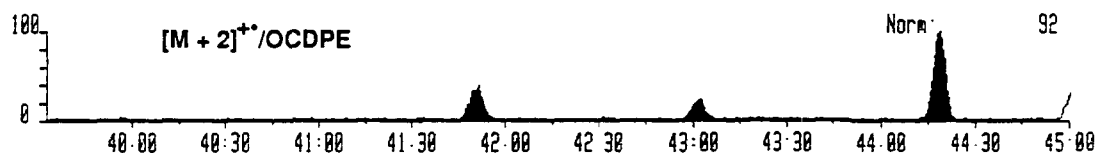
I12V2 12-SEP-89 Str Voltage 70-250S Sys DIOXINS
 Sample 1 Injection 1 Group 3 Mass 371.8230
 Text 8862A-16289 1UL INJ.



I12V2 12-SEP-89 Str Voltage 70-250S Sys DIOXINS
 Sample 1 Injection 1 Group 3 Mass 383.8642
 Text 8862A-16289 1UL INJ.



I12V2 12-SEP-89 Str Voltage 70-250S Sys DIOXINS
 Sample 1 Injection 1 Group 3 Mass 443.7584
 Text 8862A-16289 1UL INJ.



I12V2 12-SEP-89 Str Voltage 70-250S Sys DIOXINS
 Sample 1 Injection 1 Group 3 Mass 401.8559
 Text 8862A-16289 1UL INJ.

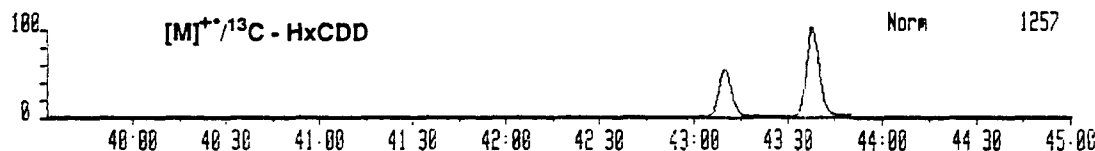
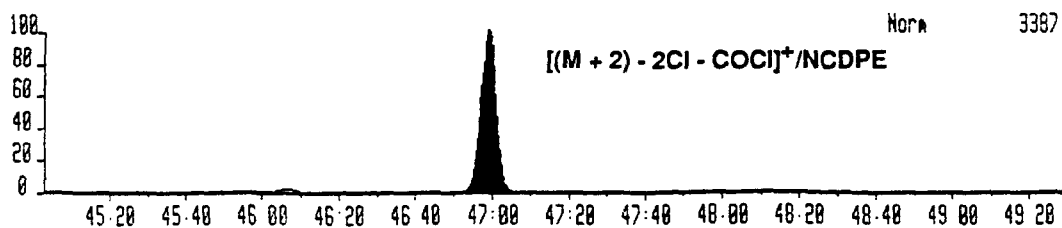
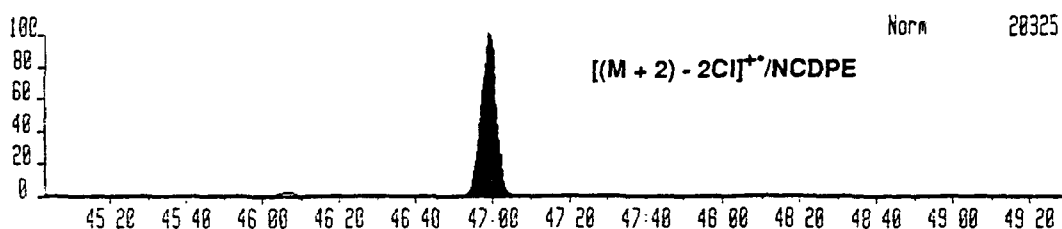


Figure 9. HRGC/HRMS-SIM mass chromatogram from the analysis of sample 16289 (ACD8700167) for OCDPE. The shaded peaks represent the responses for three OCDPEs. The response at 41:50 coelutes with 1,2,3,6,7,8-HxCDF. The response for m/z 311 and m/z 372 at 41:40 is 1,2,3,4,7,8-HxCDF.

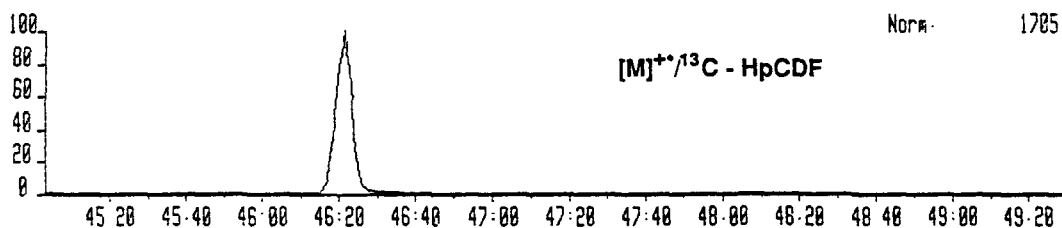
111V05 11-SEP-89 Str Voltage 70-250S Sys DIOXINS
 Sample 1 Injection 1 Group 4 Mass 344.8180
 Text 8862A-640PG/UL PCDFE STD. 1UL INJ.



111V05 11-SEP-89 Str Voltage 70-250S Sys DIOXINS
 Sample 1 Injection 1 Group 4 Mass 407.7818
 Text 8862A-640PG/UL PCDFE STD. 1UL INJ.



111V05 11-SEP-89 Str Voltage 70-250S Sys DIOXINS
 Sample 1 Injection 1 Group 4 Mass 417.8253
 Text 8862A-640PG/UL PCDFE STD. 1UL INJ.



111V05 11-SEP-89 Str Voltage 70-250S Sys DIOXINS
 Sample 1 Injection 1 Group 4 Mass 477.7196
 Text 8862A-640PG/UL PCDFE STD. 1UL INJ.

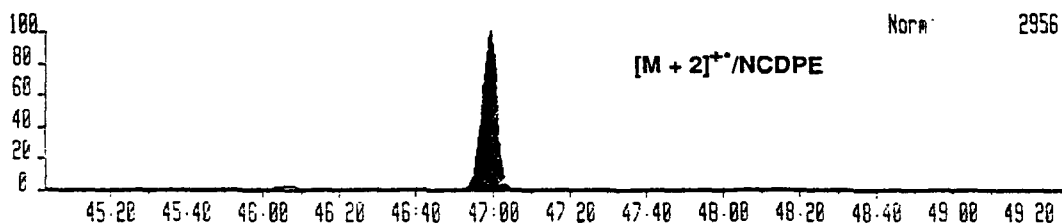
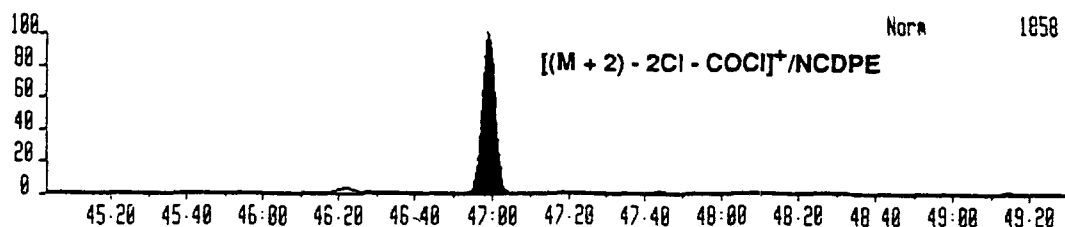
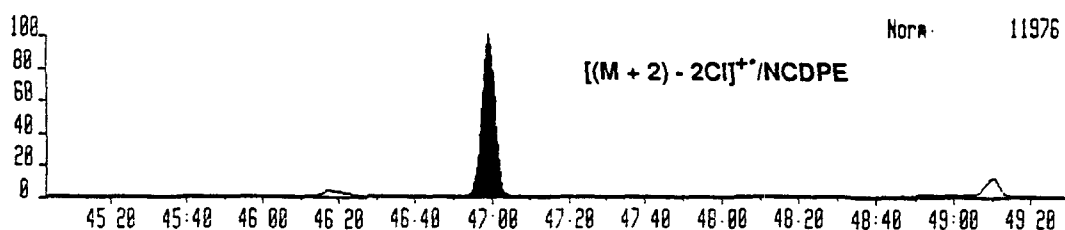


Figure 10. HRGC/HRMS-SIM mass chromatogram from the analysis of a 640-pg/ μ L standard of 2,2',3,3',4,4',5,5',6-NCDPE. The shaded peaks represent the responses for NCDPE.

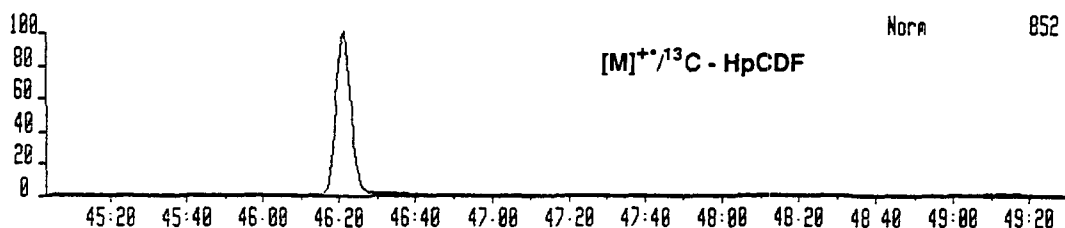
I12V2 12-SEP-89 Scr-Voltage 70-250S Sys DIOXINS
 Sample 1 Injection 1 Group 4 Mass 344.8180
 Text 8862A-16289 1UL INJ.



I12V2 12-SEP-89 Scr-Voltage 70-250S Sys DIOXINS
 Sample 1 Injection 1 Group 4 Mass 407.7818
 Text 8862A-16289 1UL INJ.



I12V2 12-SEP-89 Scr-Voltage 70-250S Sys DIOXINS
 Sample 1 Injection 1 Group 4 Mass 417.8253
 Text 8862A-16289 1UL INJ.



I12V2 12-SEP-89 Scr-Voltage 70-250S Sys DIOXINS
 Sample 1 Injection 1 Group 4 Mass 477.7196
 Text 8862A-16289 1UL INJ.

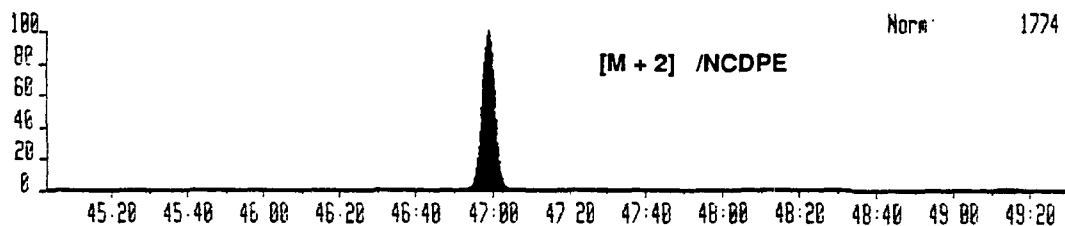
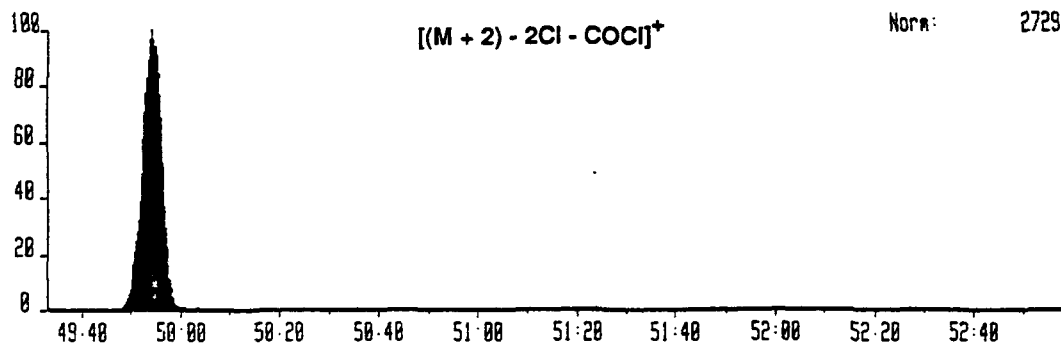
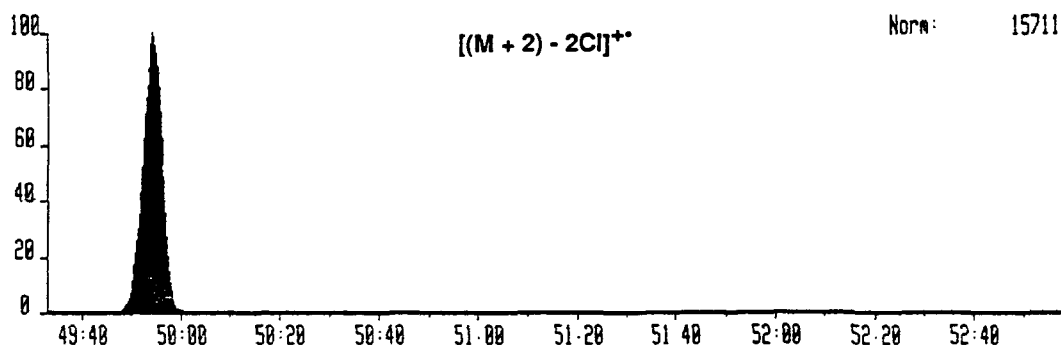


Figure 11. HRGC/HRMS-SIM mass chromatogram from the analysis of sample 16289 (ACD8700167) for NCDPE. The shaded peaks represent the NCDPE response. The response at 46:20 at m/z 345 and m/z 408 is 1,2,3,4,6,7,8-HpCDF.

I11VQ5 11-SEP-89 Str-Voltage 70-250S Sys: DIOXINS
 Sample 1 Injection 1 Group 5 Mass 378.7798
 Text:8862A-640PG/UL PCOPE STD. 1UL INJ.



I11VQ5 11-SEP-89 Str-Voltage 70-250S Sys: DIOXINS
 Sample 1 Injection 1 Group 5 Mass 441.7428
 Text:8862A-640PG/UL PCOPE STD. 1UL INJ.



I11VQ5 11-SEP-89 Str-Voltage 70-250S Sys: DIOXINS
 Sample 1 Injection 1 Group 5 Mass 511.6885
 Text:8862A-640PG/UL PCOPE STD. 1UL INJ.

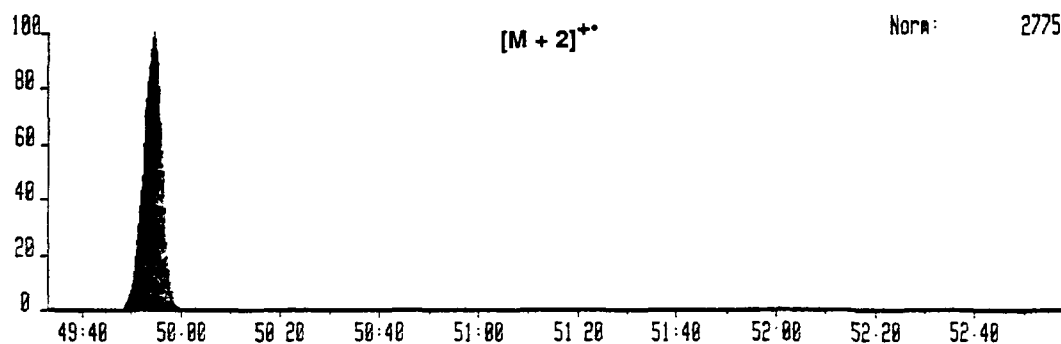
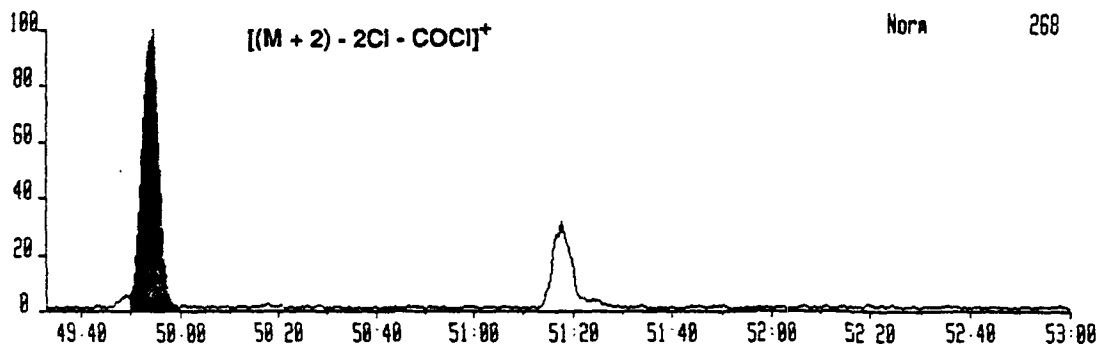
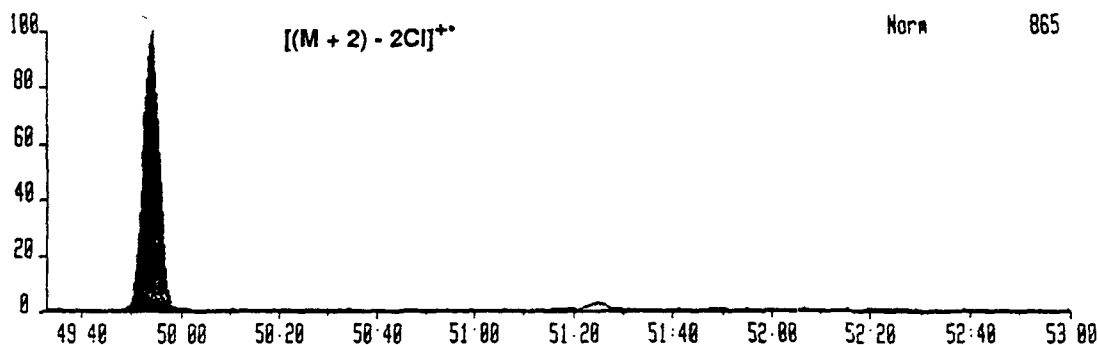


Figure 12. HRGC/HRMS-SIM mass chromatogram from the analysis of a 640-pg/ μ L standard of 2,2',3,3',4,4',5,5',6,6'-DCDPE. The shaded peaks represent the response for DCDPE.

I12V2 12-SEP-89 Sir Voltage 70-250S Sys DIOXINS
 Sample 1 Injection 1 Group 5 Mass 378.7798
 Text 8862A-16289 1UL INJ.



I12V2 12-SEP-89 Sir Voltage 70-250S Sys DIOXINS
 Sample 1 Injection 1 Group 5 Mass 441.7428
 Text 8862A-16289 1UL INJ.



I12V2 12-SEP-89 Sir Voltage 70-250S Sys DIOXINS
 Sample 1 Injection 1 Group 5 Mass 511.6885
 Text 8862A-16289 1UL INJ.

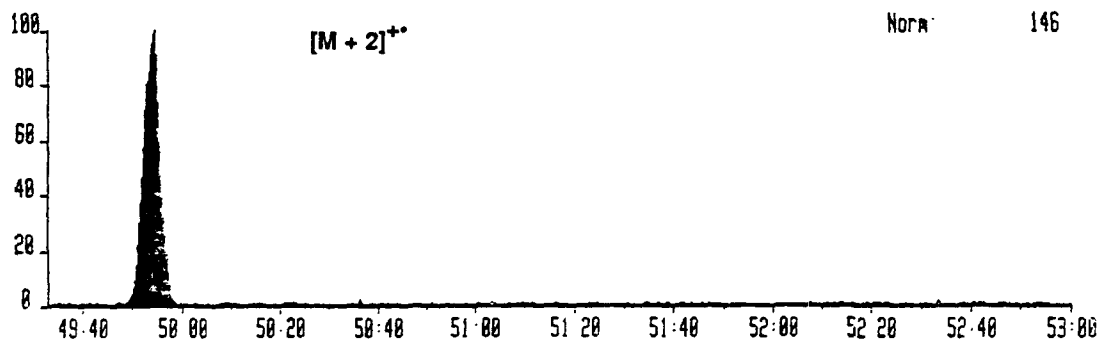


Figure 13. HRGC/HRMS-SIM mass chromatogram from the analysis of sample 16289 (ACD8700167) for DCDPE. The shaded peaks represent the DCDPE response.

C. HRGC/HRMS-SIM Detection of Polybrominated DPEs (PBDPEs)

The interpretation of the resulting mass chromatograms from the HRGC/HRMS-SIM analysis for the PBDPEs was based on the comparison of observed ratios between characteristic ions and the predicted or theoretical ratios calculated on isotopic abundances. Table 11 presents a summary of the ion ratios measured from the mass chromatograms for each sample. This table presents ion ratios within the molecular clusters and at the masses representing losses of two bromines from the molecular clusters. Comparison of characteristic ion ratios between the molecular clusters and the ion clusters from the loss of two bromines was not possible since this would have required establishing the ratios versus authentic isomers. However, the ratios of the responses that were observed demonstrated that the fragment ions characteristic of the M^+-Br_2 cluster were more intense than the M^+ cluster. This is consistent with the fragmentation pattern observed for the Bromkal solutions and with fragmentation pattern that have previously been reported in the literature for the PBDPEs.^{6,7,9} In addition, the presence of a fragment representing the combined losses of two bromines and COBr (M^+-Br_2-COBr) from the molecular cluster was also used to confirm the presence of PBDPEs. These data demonstrated that a consistent pattern of PBDPEs was detected in each sample.

As a result of the enhanced sensitivity of HRGC/HRMS-SIM, other PBDPEs in addition to the HxBDPE detected in the full scan mode were detected in the samples. In the extracts tested, brominated DPEs were observed from hexa- to decabrominated DPEs. The PBDPEs detected in the tested samples are summarized in Table 12. The NBDPE and DBDPE ions were not included in the preliminary analysis effort, but for completeness, were included in the confirmation analyses.

Because of the mixed isomer nature of the standards used for comparison, quantitation of the isomers was not possible. A commercial source of DBDPE was obtained, analyzed, and compared to the levels seen in the extracts. The levels of DBDPE in three of the five extracts were estimated to range from 400 pg/g to 700 pg/g based on the responses noted for external standard responses.

Figures 14 through 23 show examples of the extracted ion plots of the brominated DPEs detected in these samples. Shown also for comparison purposes are responses from the flame retardants, Bromkal 70-5-DE and Bromkal 79-8-DE. The shaded peaks are the PBDPEs.

A comparison of the mass chromatograms (Figure 24) from the analysis of the Bromkal standards and the adipose tissue extracts demonstrates considerable similarity in the observed response patterns for the major peak responses of each degree of bromination, especially to the OBDPE patterns.

Table 11. Theoretical vs. Measured Ion Ratios for PBDPEs in the FY87 NHATS Samples

No. of Br	Charac- teristic ions	Theo- retical ion ratio	Sample 16316 (ACD8700336)				Sample 16317 (ACD8700407)				Sample 16257 (ACD8700023)				Sample 16258 (ACD8700318)				Sample 16295 (ACD8700103) ^b				
			RT=14:54				19:24 19:40 19:56 19:55				14:56 15:33 19:24 19:40 19:55				14:51 15:24 19:21 19:36 19:52				14:51 15:25 19:22 19:37 19:52				
Hexa (6)	482/484	0.685	0.656	0.657	0.688	0.657	0.574	1.503	1.487	1.572	0.663	0.608	0.615	0.580	0.665	0.608	0.615	0.580	0.615	0.580	0.615	0.580	
	484/486	1.542	1.608	0.574	1.503	1.487	1.572	0.663	0.608	0.615	0.580	0.615	0.580	0.615	0.580	0.615	0.580	0.615	0.580	0.615	0.580		
	482/486	0.995	1.054	1.033	1.036	0.986	1.000	0.986	1.000	0.986	1.000	0.986	1.000	0.986	1.000	0.986	1.000	0.986	1.000	0.986	1.000		
	642/644	0.771	0.644	0.608 ^a	0.591 ^a	0.582 ^a	0.794	0.582 ^a	0.794	0.582 ^a	0.794	0.582 ^a	0.794	0.582 ^a	0.587 ^a	0.587 ^a	0.587 ^a	0.587 ^a	0.587 ^a	0.587 ^a	0.587 ^a	0.587 ^a	
	644/646	1.371	1.252	1.286	1.413	1.358	1.858	1.358	1.858	1.358	1.858	1.358	1.858	1.358	1.425	1.312	1.251	1.638	1.251	1.638	1.251	1.638	
Hepta (7)	560/562	0.514	W	0.359 ^a	0.384 ^a	0.364 ^a	0.369 ^a	0.364 ^a	0.369 ^a	0.364 ^a	0.369 ^a	0.364 ^a	0.369 ^a	0.364 ^a	0.379 ^a	0.389 ^a	0.379 ^a	0.389 ^a	0.379 ^a	0.389 ^a	0.379 ^a	0.389 ^a	
	562/564	1.028	W	1.106	1.024	1.092	1.038	1.092	1.038	1.092	1.038	1.092	1.038	1.092	1.025	0.996	1.025	0.996	1.025	0.996	1.025	0.996	
	560/564	0.528	W	0.397 ^a	0.394 ^a	0.398 ^a	0.383 ^a	0.398 ^a	0.383 ^a	0.398 ^a	0.383 ^a	0.398 ^a	0.383 ^a	0.398 ^a	0.388 ^a	0.388 ^a	0.388 ^a	0.388 ^a	0.388 ^a	0.388 ^a	0.388 ^a	0.388 ^a	
	719/721	0.618	W	0.535	0.532	W	0.516	W	0.516	W	0.516	W	0.516	W	0.534	0.540	W	0.540	W	0.540	W	0.540	
	721/723	1.028	W	1.227	1.220	W	1.250 ^a	W	1.250 ^a	W	1.250 ^a	W	1.250 ^a	W	0.951	0.927	W	0.927	W	0.927	W	0.927	
Octa (8)	640/642	0.771	0.882	0.816	0.834	0.820	0.795	0.862	0.840	0.832	0.868	0.826	0.887	0.877	0.852	0.833	0.751	0.826	0.838	0.811	0.772	NA	
	642/644	1.371	1.307	1.353	1.356	1.222	1.361	1.407	1.310	1.328	1.356	1.513	1.280	1.246	1.299	1.384	1.302	1.255	1.356	1.335	1.330	NA	
	640/644	1.057	1.153	1.104	1.132	1.001	1.082	0.932	1.100	1.105	1.176	1.249	1.136	1.092	1.107	1.145	1.154	1.037	1.137	1.083	1.027	NA	
	799/801	0.822	0.874	0.825	0.861	0.855	0.851	0.791	0.869	0.872	0.866	1.066 ^a	1.259 ^a	1.058 ^a	0.874	1.142 ^a	0.883	0.820	0.847	0.890	0.798	1.117 ^a	NA
	801/803	1.285	1.218	1.410	1.253	1.219	1.187	1.278	1.367	1.258	1.476	1.161	1.128	1.027	1.269	1.125	1.441	1.642	1.318	1.315	1.504	1.123	NA
Nona (9)	799/803	1.057	1.064	1.164	1.079	1.043	1.011	1.008	1.187	1.097	1.269	1.168	1.427 ^a	1.086	1.108	1.285	1.272	1.346 ^a	1.116	1.157	1.200	1.255	NA
	719/723	1.761	1.102 ^a	1.634	27:56	27:36	27:56	27:36	27:56	27:36	27:56	27:36	27:56	27:36	27:56	27:36	27:56	27:36	27:56	27:36	27:56	27:36	27:56
Deca (10)	957/959	0.857	0.931	0.765	33:10	33:08	33:10	33:08	33:10	33:08	33:10	33:08	33:10	33:08	33:10	33:08	33:10	33:08	33:10	33:08	33:10	33:08	33:10
	959/961	1.234	1.034	1.224	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W
	957/961	1.057	0.963	0.936	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W

^a = ion ratio is outside 220% window for theoretical ratio^b = Two additional weak responses of greater than 2.5 signal to noise were observed for HpBDE at retention time (RT) of approximately 21:12 and 21:56 for sample 16295

W = weak response noted but response was not integrated

NR = no response

NA = not analyzed

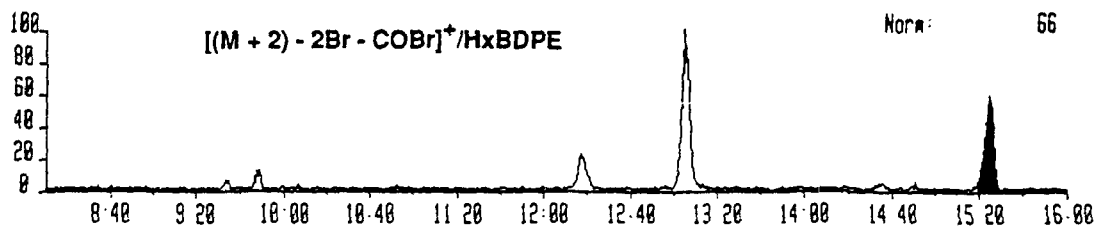
ND = not detected

Table 12. Polybrominated Diphenylether (PBDE) Detection Summary

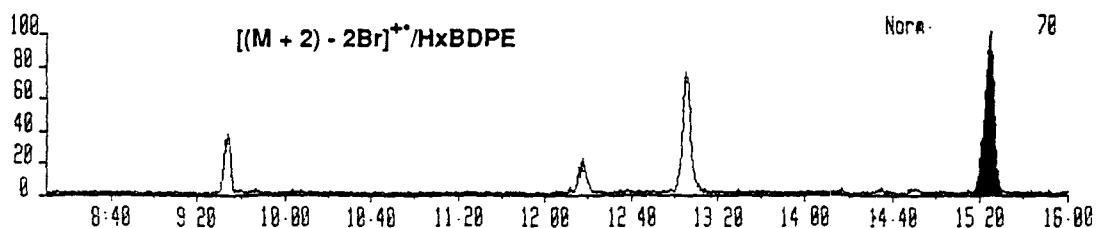
Lab ID No.	Composite No.	Batch No.	Census Division	Age Group	Number of Isomers Detected				
					HxBDDPE	HpBDPE	OBDPE	NBDPE	DBDPE
16316	ACD8700336	5	SA	15-44	1	3 (a)	5	2	1
16317	ACD8700407	5	SA	45+	2	3	5	2	1
16258	ACD8700318	1	SA	0-14	2	3	5	2	0
16257	ACD8700023	1	EN	0-14	2	3	5	2	1
16295	ACD8700103	4	EN	45+	2	5	NA (b)	NA	0

- (a) - The total number of responses for HpBDPE include peaks that were present above 2.5 times the signal-to-noise but were below the integration threshold level.
(b) - NA - Not analyzed due to insufficient sample extract.

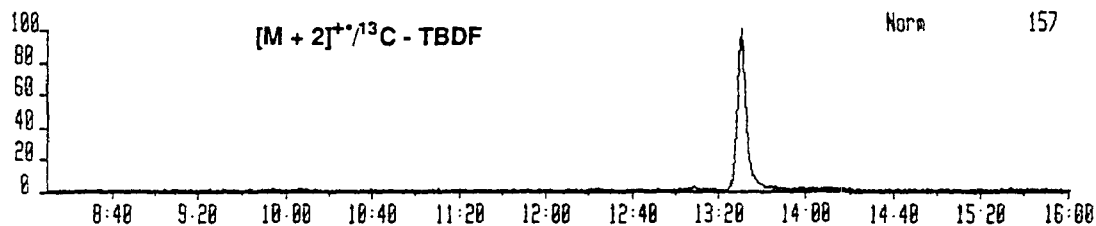
129V3 29-SEP-89 Sir Voltage 70-250S Sys OTS27
 Sample 1 Injection 1 Group 1 Mass 374.7846
 Text: BROMKAL 70-5-DE 1000PG/UL 1UL INJ.



129V3 29-SEP-89 Sir Voltage 70-250S Sys OTS27
 Sample 1 Injection 1 Group 1 Mass 481.6978
 Text: BROMKAL 70-5-DE 1000PG/UL 1UL INJ.



129V3 29-SEP-89 Sir Voltage 70-250S Sys OTS27
 Sample 1 Injection 1 Group 1 Mass 493.7381
 Text: BROMKAL 70-5-DE 1000PG/UL 1UL INJ.



129V3 29-SEP-89 Sir Voltage 70-250S Sys OTS27
 Sample 1 Injection 1 Group 1 Mass 641.5326
 Text: BROMKAL 70-5-DE 1000PG/UL 1UL INJ.

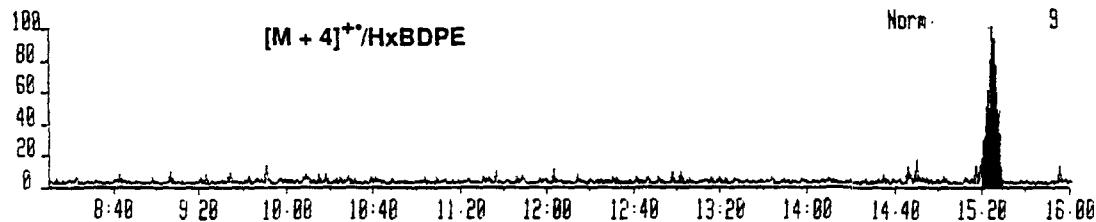
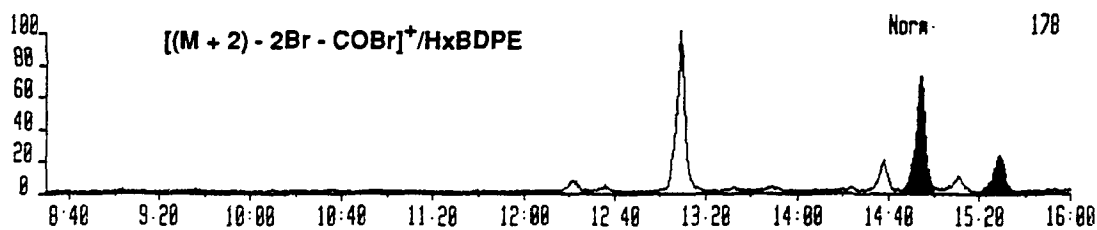
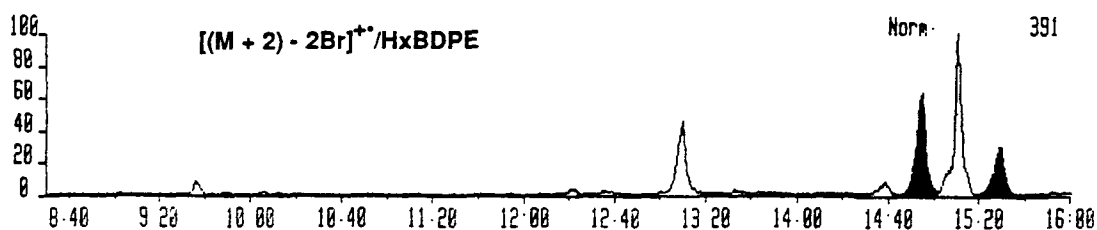


Figure 14. HRGC/HRMS-SIM mass chromatogram from the analysis of a 1000-pg/ μ L standard of Bromkal 70-5-DE for HxBDPE. The shaded peaks represent the response to HxBDPE.

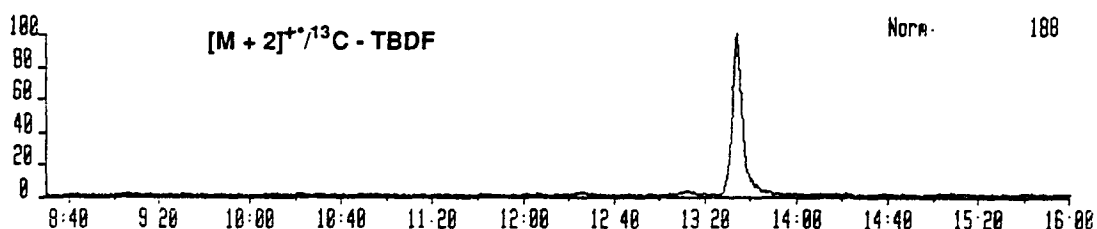
128V2 28-SEP-89 Sur Voltage 70-250S Sys OTS27
 Sample 1 Injection 1 Group 1 Mass 374.7846
 Text:8862A-16317 1UL INJ.



128V2 28-SEP-89 Sur Voltage 70-250S Sys OTS27
 Sample 1 Injection 1 Group 1 Mass 481.6978
 Text:8862A-16317 1UL INJ.



128V2 28-SEP-89 Sur Voltage 70-250S Sys OTS27
 Sample 1 Injection 1 Group 1 Mass 493.7381
 Text:8862A-16317 1UL INJ.



128V2 28-SEP-89 Sur Voltage 70-250S Sys OTS27
 Sample 1 Injection 1 Group 1 Mass 641.5326
 Text:8862A-16317 1UL INJ.

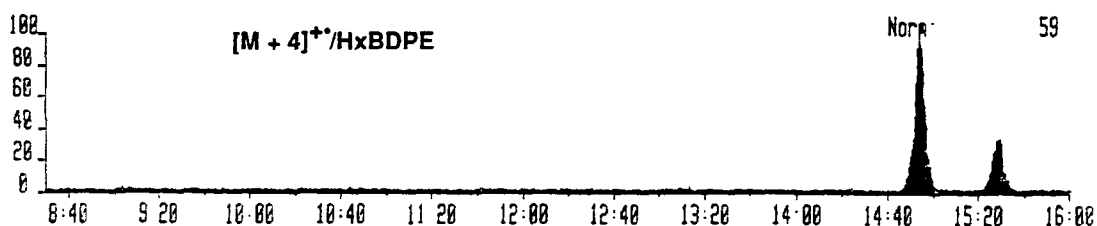
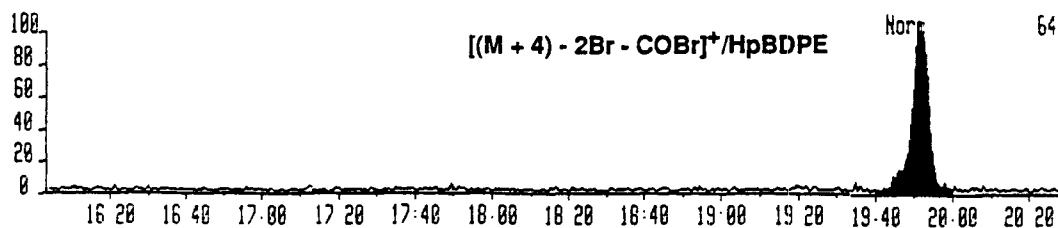
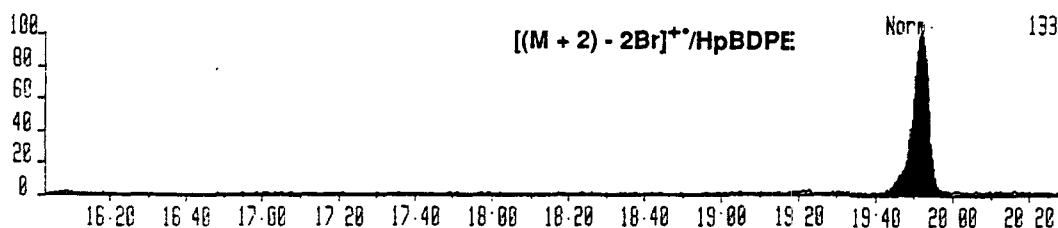


Figure 15. HRGC/HRMS-SIM mass chromatogram from the analysis of sample 16317 (ACD8700407) for HxBDPE. The shaded peaks represent the responses to HxBDPE isomers.

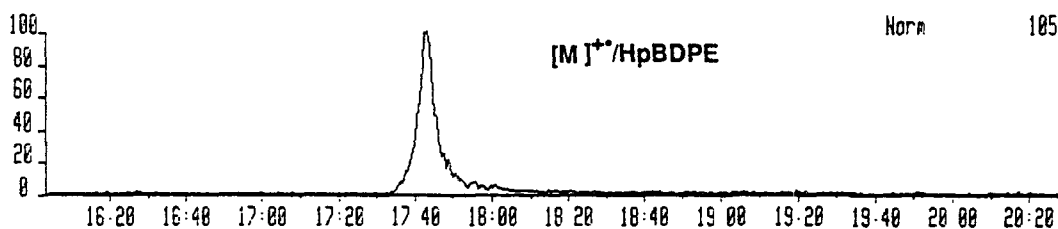
129V4 29-SEP-89 Str Voltage 70-250S Sys OTS27
 Sample 1 Injection 1 Group 2 Mass 454.6931
 Text BROMKAL 79-8-DE 1000PG/UL 1UL INJ.



129V4 29-SEP-89 Str Voltage 70-250S Sys OTS27
 Sample 1 Injection 1 Group 2 Mass 559.6004
 Text BROMKAL 79-8-DE 1000PG/UL 1UL INJ.



129V4 29-SEP-89 Str Voltage 70-250S Sys OTS27
 Sample 1 Injection 1 Group 2 Mass 573.6466
 Text BROMKAL 79-8-DE 1000PG/UL 1UL INJ.



129V4 29-SEP-89 Str Voltage 70-250S Sys OTS27
 Sample 1 Injection 1 Group 2 Mass 719.4432
 Text BROMKAL 79-8-DE 1000PG/UL 1UL INJ.

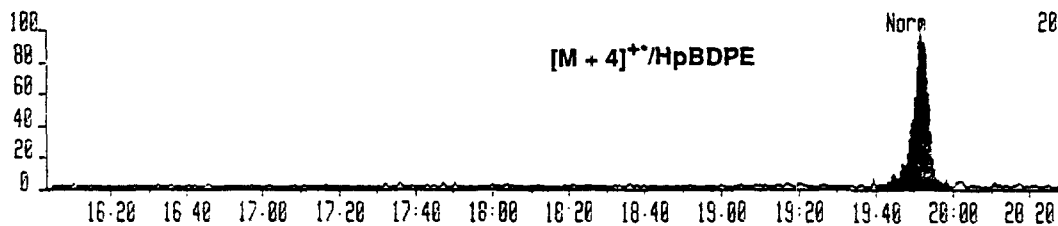
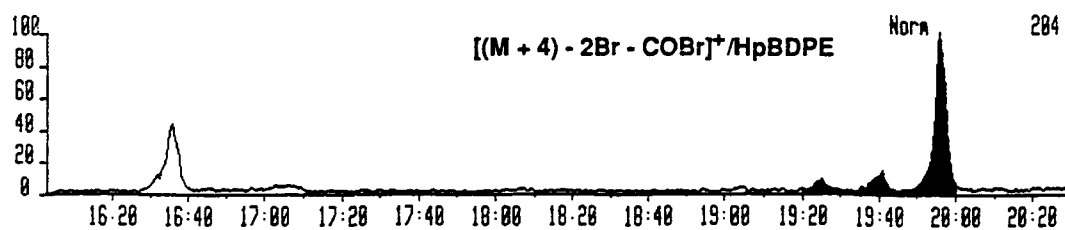
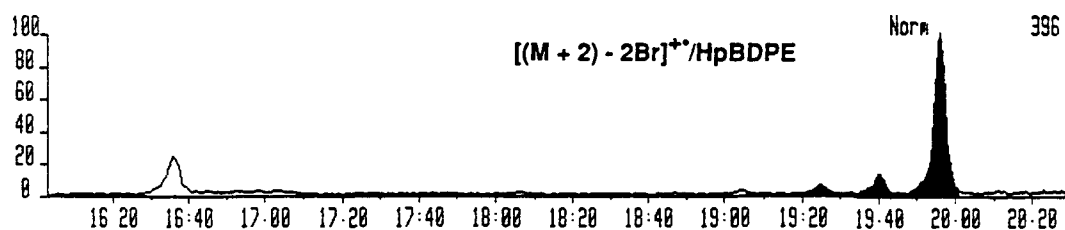


Figure 16. HRGC/HRMS-SIM mass chromatogram from the analysis of a 1000-pg/ μ L standard of Bromkal 79-8-DE for HpBDPE. The shaded peaks represent the response to HpBDPE.

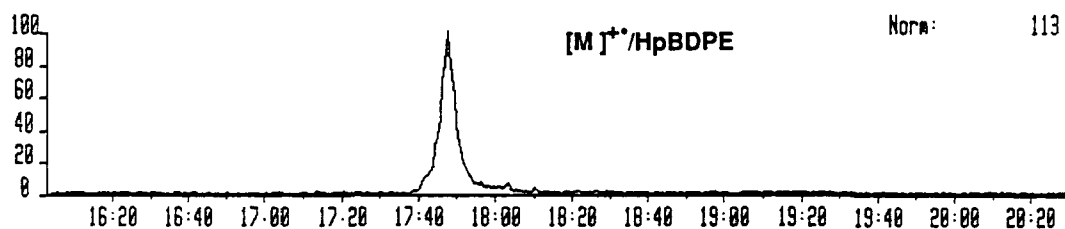
I28V2 28-SEP-89 Sur:Voltage -250S Sys OTS27
 Sample 1 Injection 1 Group 2 Mass 454.6931
 Text:8862A-16317 1UL INJ.



I28V2 28-SEP-89 Sur:Voltage 70-250S Sys: OTS27
 Sample 1 Injection 1 Group 2 Mass 559.6084
 Text:8862A-16317 1UL INJ.



I28V2 28-SEP-89 Sur:Voltage 70-250S Sys: OTS27
 Sample 1 Injection 1 Group 2 Mass 573.6466
 Text:8862A-16317 1UL INJ.



I28V2 28-SEP-89 Sur:Voltage 70-250S Sys: OTS27
 Sample 1 Injection 1 Group 2 Mass 719.4432
 Text:8862A-16317 1UL INJ.

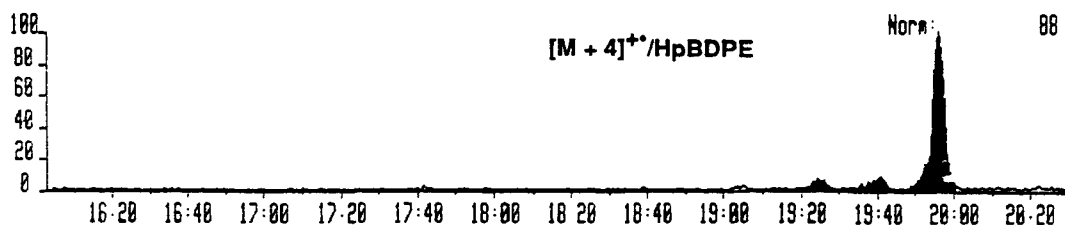
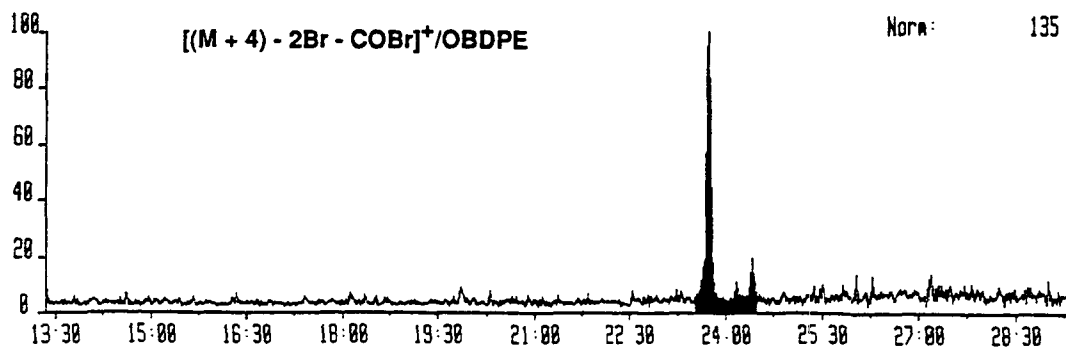
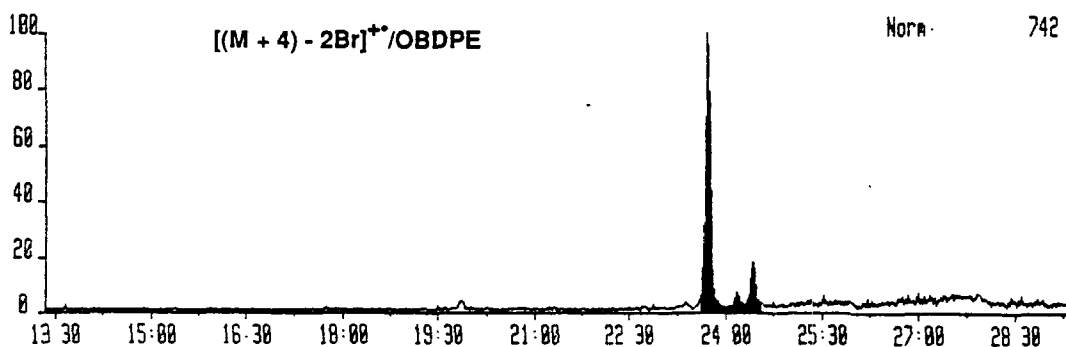


Figure 17. HRGC/HRMS-SIM mass chromatogram from the analysis of sample 16317 (ACD8700407) for HpBDPE. The shaded peaks represent the responses to HpBDPE isomers.

J03VQ2 3-OCT-89 Sir Voltage 70-250S Sys OTS27
 Sample 1 Injection 1 Group 1 Mass 532.6063
 Text BROMKAL 79-8-DE 1UL INJ.



J03VQ2 3-OCT-89 Sir Voltage 70-250S Sys OTS27
 Sample 1 Injection 1 Group 1 Mass 639.5169
 Text BROMKAL 79-8-DE 1UL INJ.



J03VQ2 3-OCT-89 Sir Voltage 70-250S Sys OTS27
 Sample 1 Injection 1 Group 1 Mass 799.3518
 Text BROMKAL 79-8-DE 1UL INJ.

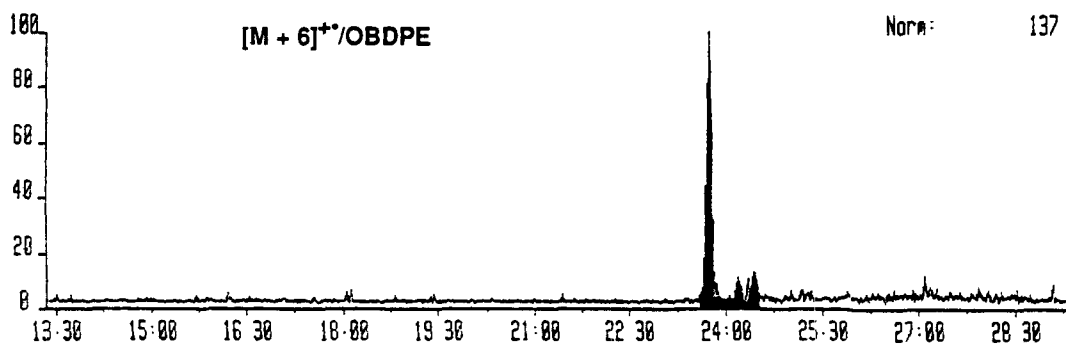
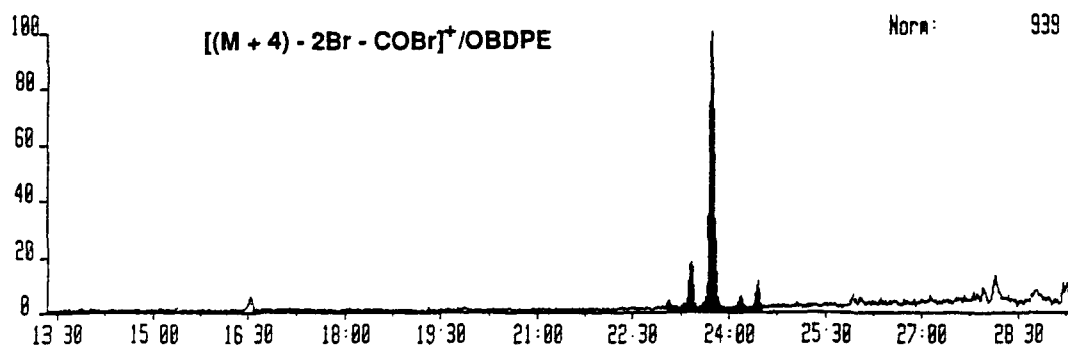
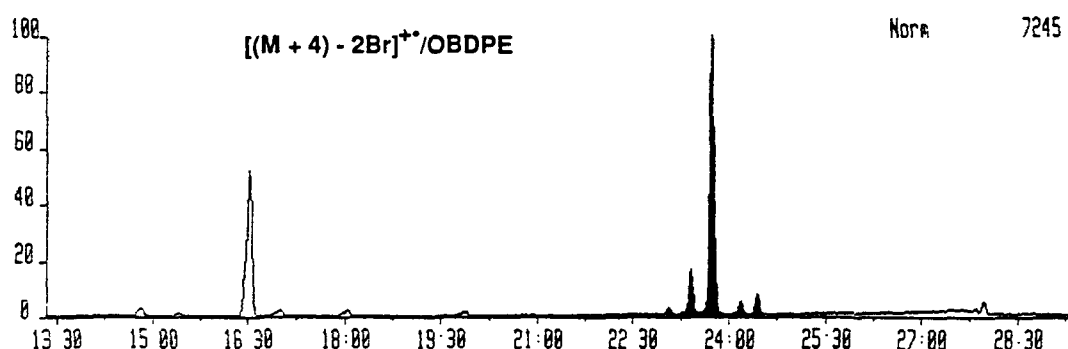


Figure 18. HRGC/HRMS-SIM mass chromatogram from the analysis of a 1000-pg/ μ L standard of Bromkal 79-8-DE for OBDPE. The shaded peaks represent the response to OBDPE.

J03V5 3-OCT-89 Scr Voltage 70-250S Sys OTS27
 Sample 1 Injection 1 Group 1 Mass 532.6063
 Text 8062A-16317 1UL INJ.



J03V5 3-OCT-89 Scr Voltage 70-250S Sys OTS27
 Sample 1 Injection 1 Group 1 Mass 639.5169
 Text 8062A-16317 1UL INJ.



J03V5 3-OCT-89 Scr Voltage 70-250S Sys OTS27
 Sample 1 Injection 1 Group 1 Mass 799.3518
 Text 8062A-16317 1UL INJ.

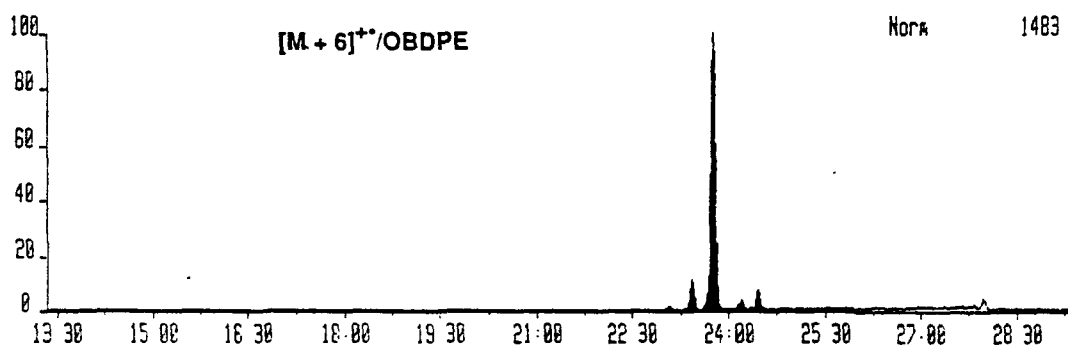
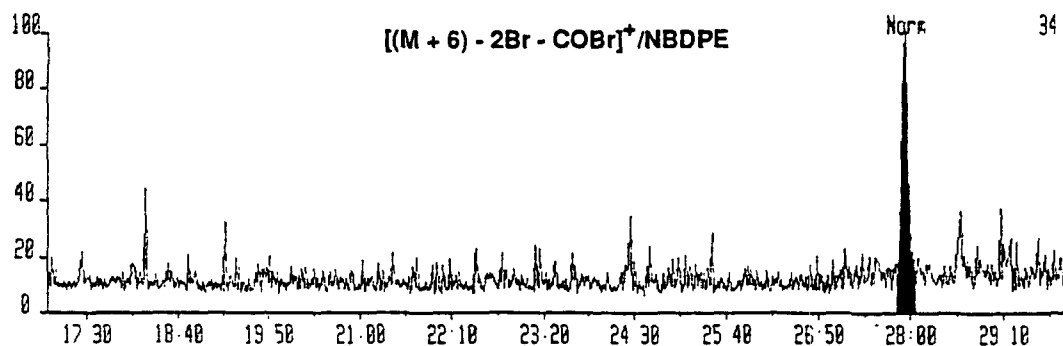
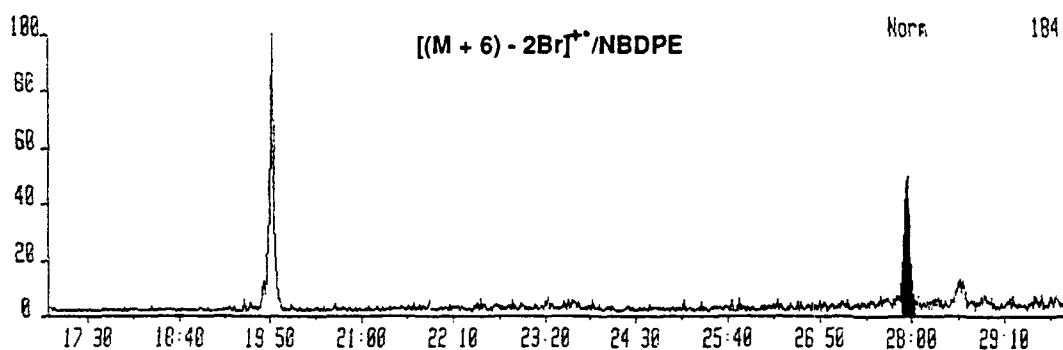


Figure 19. HRGC/HRMS-SIM mass chromatogram from the analysis of sample 16317 (ACD8700407) for OBDPE. The shaded peaks represent the responses to OBDPE isomers.

J03VQ2 3-OCT-89 Sur Voltage 70-250S Sys OTS27
 Sample 1 Injection 1 Group 1 Mass 612.5120
 Text BROMKAL 79-8-DE 1UL INJ.



J03VQ2 3-OCT-89 Sur Voltage 70-250S Sys OTS27
 Sample 1 Injection 1 Group 1 Mass 719.4250
 Text BROMKAL 79-8-DE 1UL INJ.



J03VQ2 3-OCT-89 Sur Voltage 70-250S Sys OTS27
 Sample 1 Injection 1 Group 1 Mass 877.2623
 Text BROMKAL 79-8-DE 1UL INJ.

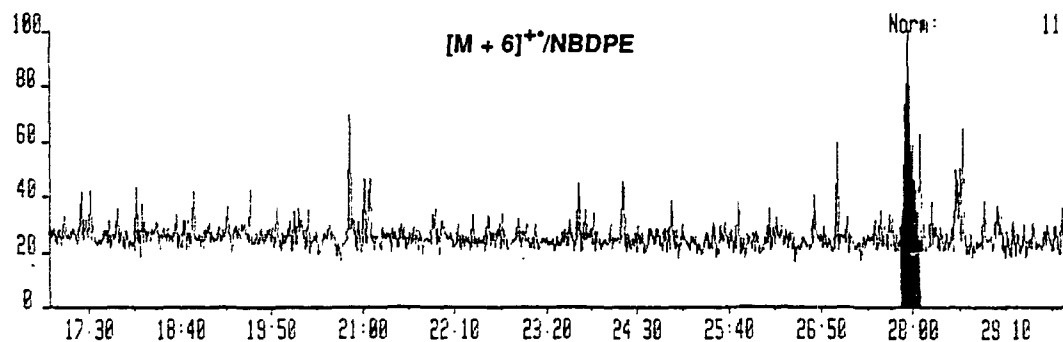
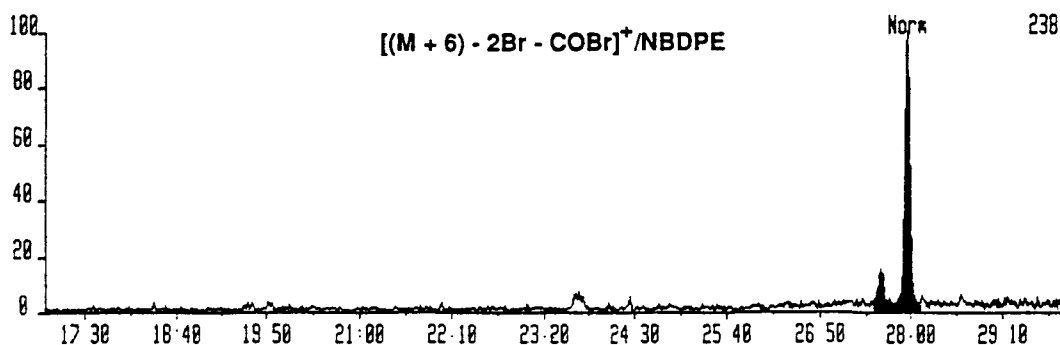
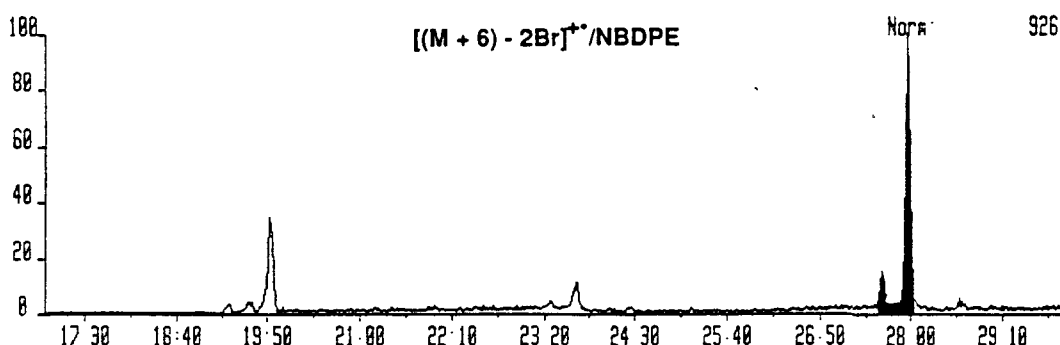


Figure 20. HRGC/HRMS-SIM mass chromatogram from the analysis of a 1000-pg/ μ L standard of Bromkal 79-8-DE for NBDPE. The shaded peaks represent the response to NBDPE.

J03V5 3-OCT-89 Str-Voltage 70-250S Sys QTS27
 Sample 1 Injection 1 Group 1 Mass 612.5120
 Text:8862A-16317 1UL INJ.



J03V5 3-OCT-89 Str-Voltage 70-250S Sys QTS27
 Sample 1 Injection 1 Group 1 Mass 719.4250
 Text:8862A-16317 1UL INJ.



J03V5 3-OCT-89 Str-Voltage 70-250S Sys QTS27
 Sample 1 Injection 1 Group 1 Mass 877.2623
 Text:8862A-16317 1UL INJ.

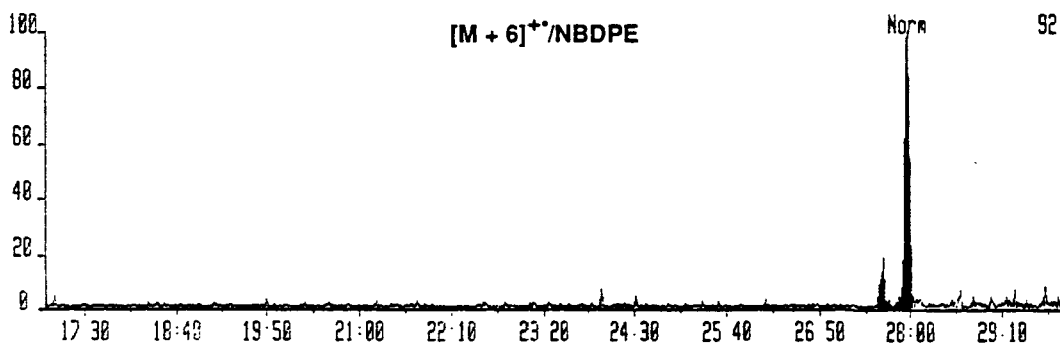
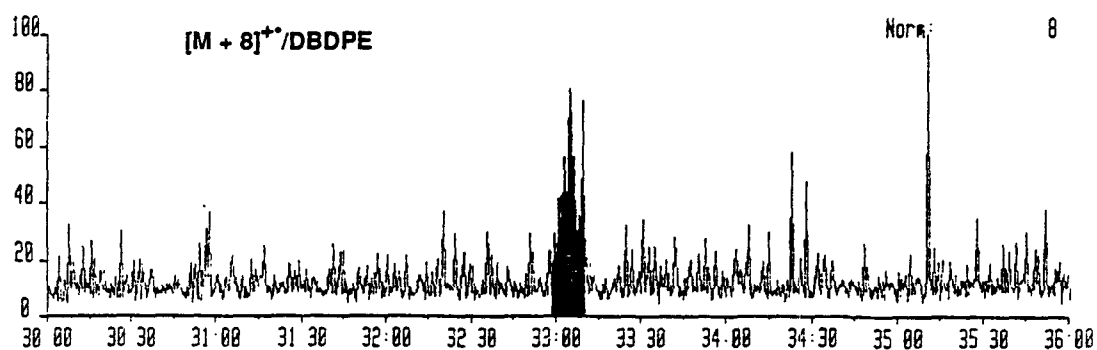
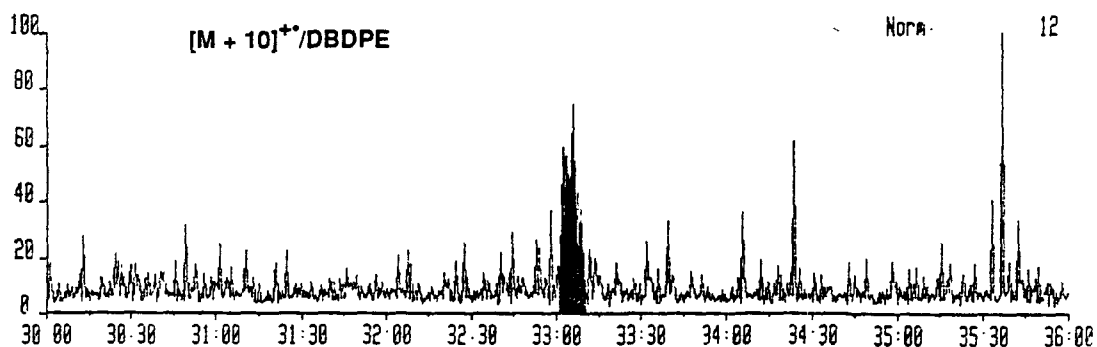


Figure 21. HRGC/HRMS-SIM mass chromatogram from the analysis of sample 16317 (ACD8700407) for NBDPE. The shaded peaks represent the responses to NBDPE isomers.

J04VQ1 4-OCT-89 Sir Voltage 70-250S Sys OTS27
 Sample 1 Injection 1 Group 1 Mass 957.1789
 Text BROMKAL 79-8-DE 1UL INJ.



J04VQ1 4-OCT-89 Sir Voltage 70-250S Sys OTS27
 Sample 1 Injection 1 Group 1 Mass 959.1690
 Text BROMKAL 79-8-DE 1UL INJ.



J04VQ1 4-OCT-89 Sir Voltage 70-250S Sys OTS27
 Sample 1 Injection 1 Group 1 Mass 961.1670
 Text BROMKAL 79-8-DE 1UL INJ.

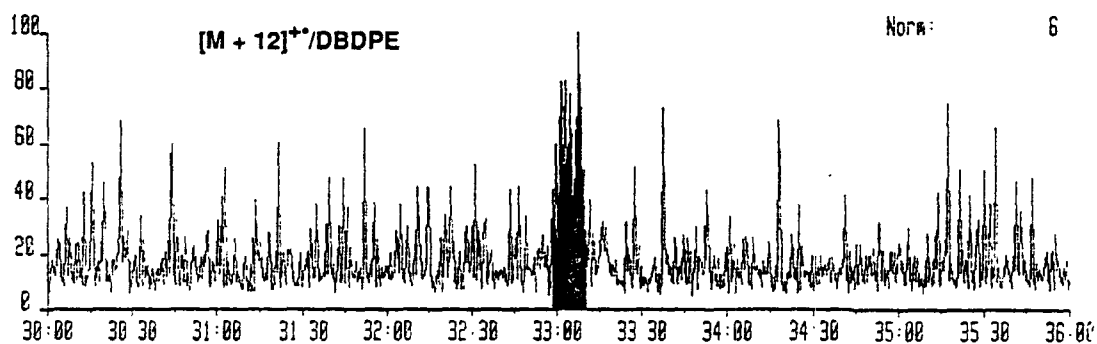
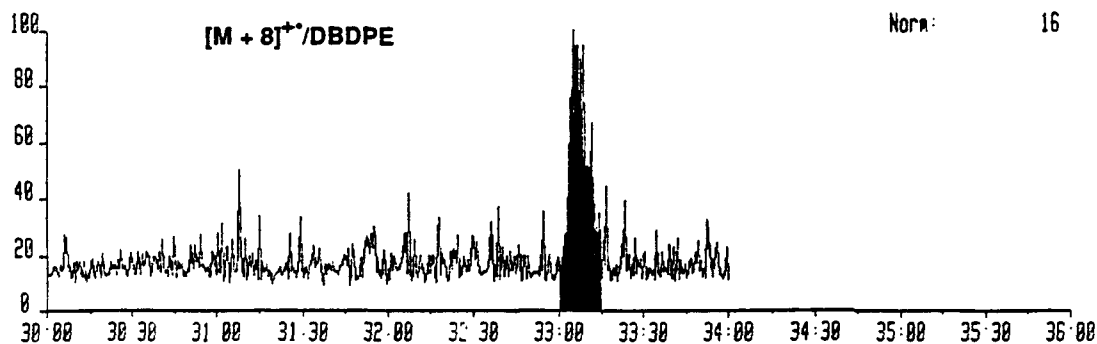
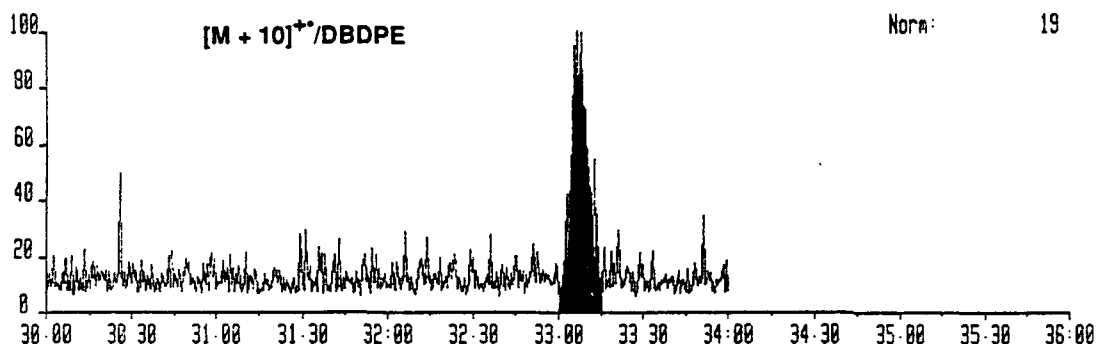


Figure 22. HRGC/HRMS-SIM mass chromatogram from the analysis of a 1000-pg/ μ L standard of Bromkal 79-8-DE for DBDPE. The shaded peaks represent the response to DBDPE.

J04V6 4-OCT-89 Str Voltage 70-250S Sys OTS27
 Sample 1 Injection 1 Group 1 Mass 957.1789
 Text 8862A-16317 1UL INJ.



J04V6 4-OCT-89 Str Voltage 70-250S Sys OTS27
 Sample 1 Injection 1 Group 1 Mass 959.1698
 Text 8862A-16317 1UL INJ.



J04V6 4-OCT-89 Str Voltage 70-250S Sys OTS27
 Sample 1 Injection 1 Group 1 Mass 961.1670
 Text 8862A-16317 1UL INJ.

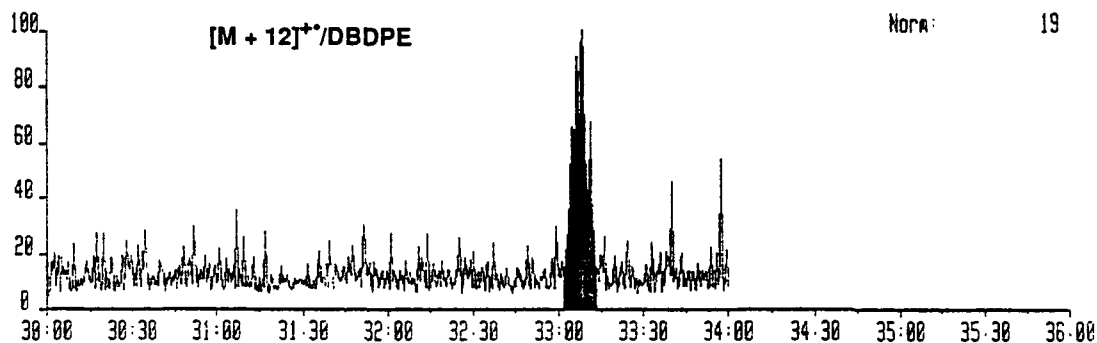
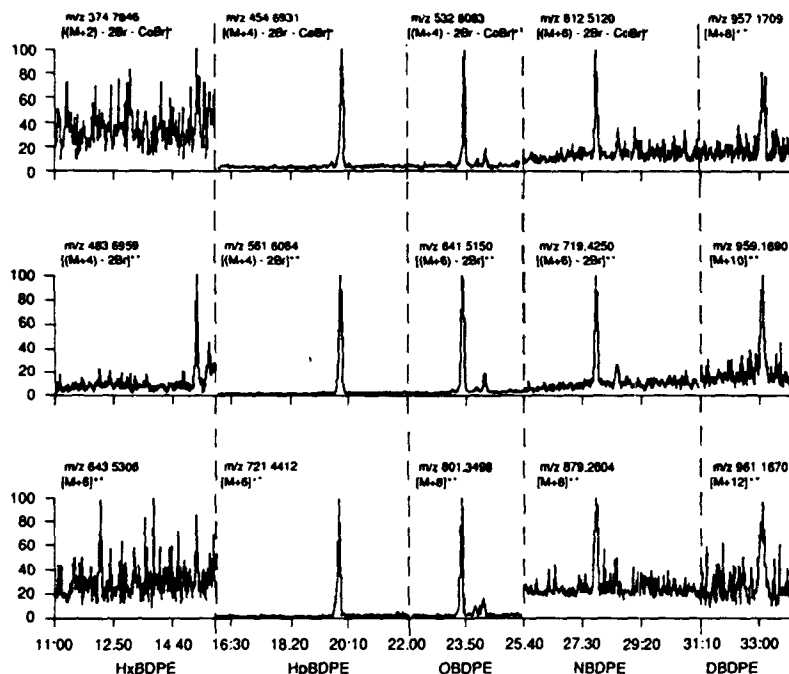
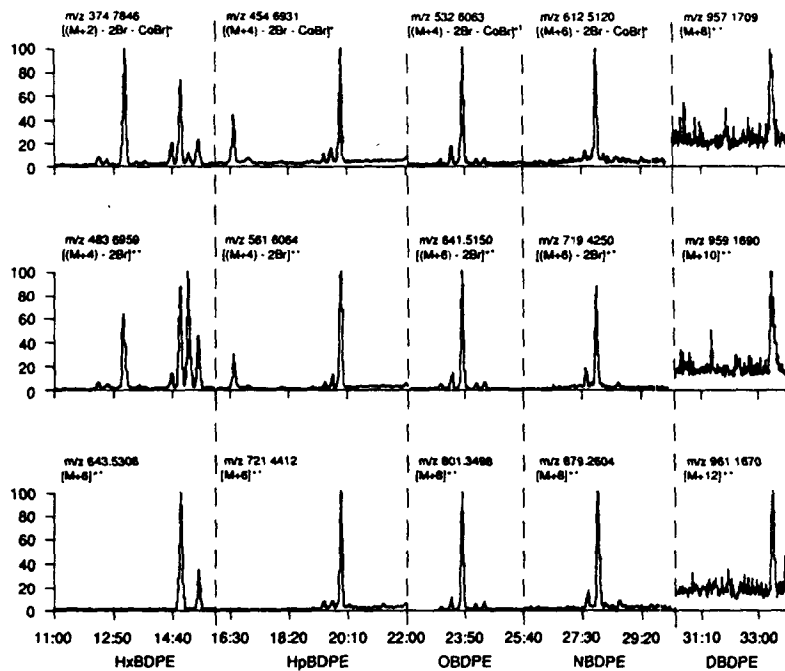


Figure 23. HRGC/HRMS-SIM mass chromatogram from the analysis of sample 16317 (ACD8700407) for DBDPE. The shaded peaks represent the responses to DBDPE.



HRGC/HRMS-SIM Mass Chromatogram from the Analysis of A Solution of Bromkal



HRGC/HRMS-SIM Mass Chromatogram from the Analysis of An FY87 NHATS Sample

Figure 24. Composite mass chromatograms for comparison of PBDPEs between Bromkal standards (70-5-DE and 79-8-DE) and an FY87 NHATS sample

The mass chromatograms for the HxBDPE and HpBDPE indicate the presence of additional brominated compounds. These responses were noted for the ion cluster representing the loss of two bromines and the loss of COBr. The masses and ratios of ions observed are consistent with either tetra- or pentabromo-DPEs or TBDF or PeBDF. The responses do not overlap with the 2,3,7,8-TBDF or 1,2,3,7,8-PeBDF which were evaluated in the previous study. Most of these responses are detected in Bromkal 70-5-DE, which has been reported to contain 41.7% 2,2',4,4'-TBDE, 7.6% assorted PeBDPEs, 44.4% 2,2',4,4',5'-PeBDPE, and 6% assorted HxBDPEs.^{6,11}

Previous efforts for the analysis of PBDFs from plastics and resins treated with brominated fire retardants have reported that the overlap of PBDFs with PBDPEs of one additional degree of bromination may be more predominant than two additional bromines (i.e., the PeBDPE homolog may overlap more with TBDF than does HxBDPE).^{5,14} Most of the responses from the Bromkal standards are detected in the samples, suggesting the presence of TBDEs and PeBDPEs rather than PBDFs. Other researchers have identified PBDPE patterns similar to the Bromkal pattern in fish and avian tissues and eggs from the United States and Canada, Sweden, and Japan.¹²⁻¹⁷

The analyses of the laboratory method blanks prepared along with the NHATS samples demonstrated that the laboratory was free of background levels of these brominated compounds.

VI. CONCLUSIONS

This study has resulted in the detection and confirmation of PHDPEs in adipose tissue. The identifications are based on both full scan mass spectrometry and HRMS-SIM ($R > 10,000$), comparison of observed responses versus standards, comparison of theoretical ion ratios versus observed ion ratios for characteristic ions, and measurements of fragment losses from the molecular ion clusters.

The full scan analysis demonstrated the presence of the hexabromo-DPE (HxBDPE) and the nonachloro-DPE (NCDPE). These compounds were observed as the major responses in the adipose tissue extract and correspond to concentrations that were estimated to exceed the 1 ng/g (ppb) level.

The estimates of the PCDPE levels from the preliminary analysis effort, which focused on the determination of PCDFs, are comparable with the values calculated in this confirmation study versus authentic PCDPE standards. Hence, the data (Table I) generated versus the PCDF RRF measurements are considered good preliminary values of the levels in the general U.S. population. The levels of PCDPEs reported have a number of caveats: the sample preparation procedures are not optimized for PCDPE recoveries and the quantitations are based on recoveries of carbon 13-labeled PCDF internal quantitation standards. The presence of the PBDPEs was confirmed by the additional HRMS-SIM experiments, although it was not possible to refine the estimates of concentrations from the preliminary effort due to a lack of individual PBDPE isomers.

In addition to confirming the presence of the hexa- through octabromo DPEs, which were detected in the analysis for PBDFs, the nona- and decabromo-DPEs were identified in the adipose tissue extracts. The decabromo-DPE response was estimated to range from ND to 700 pg/g based on an external standard measurement using a solution containing only the decabromo-DPE. This compound was detected in three of the five extracts analyzed in this confirmation effort.

The PCDPs are usually associated with PCDD/PCDF as by-products in the production of chlorinated pesticides, wood preservatives, and other commercial products. Therefore, their presence in adipose tissue provides additional indication of human exposure to such commercial products and, ultimately, PCDDs and PCDFs.

The PBDPEs are primarily used as fire retardants and are used extensively in the plastics, clothing, building, and numerous other industries. Their presence in human adipose tissue and the similarity of the major peak patterns between the tissue samples and the fire retardant mixed standards suggests exposure to these compounds from commercial products.

VII. RECOMMENDATIONS FOR FURTHER STUDY

Further evaluation of the levels of halogenated aromatic compounds in human adipose tissues should include the refinement of the analytical methods to promote simultaneous determination of dioxins, furans, diphenyl-ethers, and non-ortho-substituted biphenyls. This approach would be especially useful in studies focused on chlorinated aromatics. The resulting data from these studies should be analyzed for correlations between compound classes to provide indications of routes of exposure. Refinement of the analytical methods will require development and acquisition of additional analytical standards and internal quantitation standards to promote quantitative recovery and precision.

Additional analysis efforts for PBDPEs should include experiments to determine the presence of other PBDPEs such as the tetra- and pentabromo congeners.

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<p>16. Abstract (Limit: 200 words)</p> <p>This study has resulted in the detection and confirmation of polyhalogenated diphenylethers (PHDPEs) in human adipose tissues. The identifications are based on both full scan and selected ion monitoring (SIM) high resolution mass spectrometry (HRMS) analysis efforts. The samples that were analyzed were selected from composites of the fiscal year 1987 (FY87) National Human Adipose Tissue Survey (NHATS) repository. This specific analysis effort to confirm the presence of the PHDPEs was conducted as a result of observations of response to these compounds during the analysis of the FY87 NHATS composites for polyhalogenated dibenzo-p-dioxins and dibenzofurans. The PHDPEs are of interest as a result of their planar aromatic structure and potential toxicological properties. The PHDPEs are commercially produced as brominated fire retardants and are known contaminants in other commercial products such as pentachlorophenol.</p> <p>The concentrations of the PHDPEs were estimated from the preliminary analysis for the polyhalogenated dibenzo-p-dioxins and dibenzofurans. The analysis efforts summarized in this report were conducted using available PHDPE standards. Identification of the PHDPEs was based on (1) the comparison of full scan mass spectra in the samples versus authentic standards and (2) the application of SIM techniques to compare theoretical ion ratios versus observed ion ratios for characteristic ions and measurement of fragment losses from the molecular ion clusters.</p>			
<p>17. Document Analysis a. Descriptors</p> <p>Polyhalogenated diphenylethers, PHDPEs; Polyhalogenated brominated diphenylethers, PBDPEs; Polyhalogenated chlorinated diphenylethers, PCDPEs; Polyhalogenated dibenzo-p-dioxins, PHDDs; Polyhalogenated dibenzofurans, PHDFs; Human adipose tissue; National Human Adipose Tissue Survey, NHATS</p> <p> b. Identifiers/Open-Ended Terms</p> <p>High resolution mass spectrometry confirmation</p> <p> c. COSATI Field/Group</p>			
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